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ERRATA AND AUTHORS' EMENDATIONS

Page 48, third line from bottom, "distilled" should be "each mixed."
Page 99, figure 2, *B*, "Ward City" should be "Ward Creek."
Page 100, sixth line, "Humboldt Range" should be "Humboldt Basin."
Page 120, seventeenth line, "horizontal" should be "elevation."
Pages 125 and 126, the legend of figure 9 belongs to figure 10 and the legend of figure 10 belongs to figure 9.
Page 235, footnote 1, "University of Kansas" should be "University of Arkansas."
Page 252, nineteenth line, "vitamin G" should be "vitamin B."
Page 318, reference 2, "Holz-spund" should be "Holz-und", and reference 12, "Azetyl-spund" should be "Azetyl-und."
Page 344, legend for figure 2, "*irregulare*" should be inserted after "*sigmoideum*."
Pages 388-396, "whitefish meal" wherever used should be "white fishmeal."
Page 389, lines 33-35, figures should align, 89
81, 108, and 79
96 88
Page 390, second heading at bottom of page should be "50 percent protein grade" instead of "55 percent protein grade."
Page 396, third line, "haddock meals" should be "white fishmeals."
Page 396, seventh line from bottom, "solvent" should be "expelier."
Page 402, first line under Experimental Procedure should read "Corn-gluten meal, linseed meal, and soy-bean-oil meal were each substi-."
Page 430, table 1, third line, last column, "3.700" should be "3.300."
Page 430, table 1, next to bottom line under heading "basal area by-", "7.4128, 7.398, 7.392, and 7.412" should be "0.4128, 0.398, 0.392, and 0.412", respectively.
Page 454, legend for plate 2, all magnifications should be reduced 25 percent.
Page 522, first line, "Elsinoä" should be "Elsinoë."
Pages 721 and 724. Figure 1 should be figure 3 and figure 3 should be figure 1.
Page 739, ninth line from bottom, "about 2 to 1,000 dilution" should be "about 1-2000 dilution."
Page 784, twenty-eighth line, delete the words "exterior surfaces of the."
Page 794, sixth line from bottom, "plates 8" should be "plate 8."
Page 833, table 3, second line, omit "Petals narrow, margins crimped—Continued."
Page 864, legend for figure 7, *B*, magnification should be "403" instead of "203."
Page 876, figure 15, *A*, "*ii*" should be "*ii*."
Page 925, second line from bottom, "an apple" should be "on apple."
Page 1002, legend for figure 12, B, should read "section from a primary lateral illustrating the origin of a very small branch, shown about natural size."

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VOL. 51 WASHINGTON, D. C., JULY 1, 1935 NO. 1

THE EFFECTS, ON CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS, OF FEEDING LOW-CALCIUM RATIONS FOR LONG PERIODS[1]

By EDWARD B. MEIGS, *senior physiologist*, WILLIAM A. TURNER, *associate chemist*, EDWARD A. KANE, *assistant chemist*, and LEO A. SHINN, *junior chemist, Division of Dairy Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture*[2]

INTRODUCTION

A study of the numerous experiments in which calcium and phosphorus balances of dairy cows have been determined during the last 25 years gives a confusing impression. Some of the experiments indicate that cows readily lose these elements from their bodies, and are likely to lose them at a rapid rate if they are giving any considerable quantity of milk. Thus, Hart, McCollum, and Humphrey (*6*)[3] obtained results in 1909 which would indicate that a cow giving from 12 to 18 kg of milk daily and fed on rations low in calcium lost about 2 kg of calcium from her body in the course of 111 days, or more than 25 percent of all the calcium her body might have been expected to contain. Since then the calcium and phosphorus metabolism of dairy cows has been studied in many experiments at several American experiment stations. The results of the earlier experiments at Beltsville, Md. (*15*), and of later ones at the Wisconsin (*7*) and Beltsville (*28*) Stations indicate (1) that milking cows are likely to be losing calcium from their bodies even when their rations contain large amounts of calcium, (2) that on rations low in calcium, the losses are likely to be larger, and (3) that it is probably difficult to keep cows in calcium equilibrium when they are subjected to the usual dairy regime. The practical conclusion from such results is, of course, that it is wise to feed liberally milking cows on rations that contain large amounts of calcium.

On the other hand, certain recent work at the Michigan (*12*) and Vermont (*4*) Stations indicates that mature cows which are fed for several years on rations low in calcium may readily assimilate enough calcium from such rations to supply what is needed for their milk, and may sometimes even be storing considerable amounts of it in their bodies. Some results of the Vermont work indicate also that calcium and phosphorus may be stored in proportions very different from those in which they are usually found in mammalian bone or in the mammalian body.

[1] Received for publication Mar. 7, 1935; issued August 1935.

[2] The writers acknowledge the help given by P. E. Howe, O. G. Hankins, N. R. Ellis, and other members of the Bureau of Animal Industry, U. S. Department of Agriculture. The work would have been difficult or impossible without the advice and help received from them on all the details of the slaughter analyses.

[3] Reference is made by number (italic) to Literature Cited, p. 24.

In carrying out balance experiments which extend over long periods it is not easy to avoid small errors in sampling and making analyses which might accumulate in the course of the experiment and appear in the final results as large distortions of the total quantities of calcium and phosphorus assimilated. Some of the Vermont results suggest strongly to anyone familiar with the usual proportions of calcium and phosphorus in body and bone that such errors have occurred. It seems possible also that such errors may partly explain the surprisingly large losses of calcium and phosphorus that appear to have occurred in other balance experiments.

It does not seem justifiable, however, to discard on mere suspicion the results of the great amount of work represented by balance experiments. Fortunately, analyses of the whole bodies of cattle provide a satisfactory though laborious method of checking many aspects of the balance experiments. There already exists a large amount of information which throws much light on the limits of variability in the normal calcium and phosphorus content of the bovine body and bone.

In the present study the results of previous body analyses of cattle have been used in conjunction with the results of new experiments to make a beginning toward showing how far the normal quantities of calcium and phosphorus in body and bone are likely to be altered by subjecting the animals to long periods on rations low in calcium.

OUTLINE OF STUDY

The experimental work to be described and discussed may be divided into three groups.

In the first group the calcium intake in the feed and the calcium output in the milk and calves of cows was recorded over long periods, at the end of which the animals either died naturally or were slaughtered. Analyses were then made of several of the bones of the cows, and a rough attempt was made to estimate whether the feeding of rations low in calcium had any tendency to reduce the weight of the bones in relation to that of the rest of the body.

But, as this method of estimating changes in the calcium and phosphorus content of the body is unsatisfactory for many reasons, a second group of experiments was carried out in which the intake of calcium in the feed and the output through the milk and calves of three cows over periods of several years was determined. Two of these cows were on rations containing liberal proportions of alfalfa hay and the third was on a ration of grain with timothy hay as the sole roughage. At the end of the experimental period the three cows were slaughtered, and determinations were made of the calcium, phosphorus, fat, nitrogen, ash, and water in their entire bodies.

A third group of experiments consisted in determining the calcium and phosphorus balances of cows which had been on low-calcium rations for over a year, and were still on low-calcium rations at the time the balances were determined.

PREVIOUS BODY ANALYSES OF CATTLE

Determinations of fat, calcium, phosphorus, and other mineral elements in the bodies of cattle were carried out many years ago by Lawes and Gilbert of Great Britain (*14*), and much more recently by

Trowbridge, Moulton, Haigh, Hogan, and others at the Missouri Agricultural Experiment Station (*5*, *11*, *16*, *17*, *18*, *19*, *23*). In the Missouri investigation the animals were fed in various ways, though not in ways that would be expected to produce any very marked changes in the calcium and phosphorus content of the body. A study of the results, however, throws much light on the relationship between the calcium and phosphorus content of the bodies of cattle, and on the variations which are to be expected in the calcium, phosphorus, and fat content at different ages and under different conditions of feeding.

The American investigations were much more extensive than the British ones and will be considered first. Hogan and Nierman (*11*) give figures for analyses of a few embryos, a number of calves slaughtered at various ages from just after birth to 8 months old, and a number of steers about 4 years old. The animals were fed on rations which were generally similar in make-up to rations fed under good conditions of farm practice, and all contained fairly liberal proportions of calcium. Three planes of nutrition were used, however, for the study; the first, high enough to permit marked fattening; the second, sufficient to permit good growth without much fattening; and the third, so low that growth as well as fattening was considerably restricted. The results show that while the body fat varies greatly according to the plane of nutrition, the percentages of calcium and phosphorus in the fat-free bodies of the animals are little affected. This point is brought out by the figures in table 1. The thin animals have only about one-third as much fat in their bodies as the fat ones, but about 1.6 times as much calcium and phosphorus. The calcium and phosphorus content of the fat-free bodies, on the other hand, varies comparatively little, there being less than 10 percent difference between the highest and the lowest figures for either element.

TABLE 1.—*Average calcium, phosphorus, and fat in the whole bodies and in the fat-free bodies of steers from 40 to 48 months old fed at different planes of nutrition*[1]

Plane of nutrition	Calcium	Phosphorus	Fat
	IN WHOLE BODIES		
	Percent	*Percent*	*Percent*
High	0.98	0.53	44.21
Medium	1.51	.80	20.43
Low	1.59	.84	14.66
	IN FAT-FREE BODIES		
High	1.75	0.94	----------
Medium	1.90	1.01	----------
Low	1.86	.99	----------

[1] The figures for calcium and phosphorus are from Missouri Research Bulletin 107 (*11*); those for fat are from Missouri Research Bulletin 55 (*18*).

The manner in which the calcium and phosphorus content changes with advancing age in the fat-free bodies and in the fat-free soft tissues of cattle, as brought out by calculations from the Missouri data, is shown in table 2.

TABLE 2.—*Calcium and phosphorus in fat-free bodies and fat-free soft tissues of cattle at various ages, as calculated from Missouri data*

FAT-FREE BODIES [1]

Kind, and age of animal	Calcium	Phosphorus
	Percent	*Percent*
Fetus, 232 days old	0.879	0.493
New-born calves (average of 5)	1.241	.708
Steers (average of 9, 3 to 8 months old)	1.453	.840
Steers (average of 9, 40 to 48 months old)	1.838	.978

FAT-FREE SOFT TISSUES [2]

Steers (average of 3):		
3 months old	0.022	0.186
5 months old	.022	.170
8 months old	.021	.159
40 months old	.016	.140
44 months old	.016	.144
48 months old	.017	.143

[1] Calculated from Missouri data (*11*).
[2] Calculated from Missouri data (*11, 18*).

Table 2 shows that the percentage of calcium and of phosphorus in the fat-free bodies of cattle tends to increase materially up to the period of maturity. Experiments like those which are to be described in this paper should, therefore, be confined to mature cattle. Table 2 also shows that the percentage of calcium and of phosphorus in the fat-free soft tissues of cattle is small and fairly constant, but that both the calcium and phosphorus tend to decrease with advancing age.

Still other points of interest brought out by the figures in table 2 are that the calcium content of the soft tissues of cattle is very small; that the phosphorus content is larger, but still only a rather small fraction of that contained in the bones; and that the ratio between the calcium and phosphorus contained in the whole body tends to be fairly constant. The calcium contained in the soft tissues of mature steers is less than 1 percent of that contained in the whole body, while the phosphorus in the soft tissues is about 15 percent of the total. The approach to constancy of the Ca/P ratio in the whole body is dependent on the still more nearly constant Ca/P ratio in bone. In the whole series of animals whose bone analyses are given by Hogan and Nierman (*11*), the Ca/P ratio did not fall below 2:1 or rise above 2.14:1 for the skeleton of any single animal. These figures are a little lower and a little less constant than they might be because of the fact that the skeletons were only roughly cleaned. In the carefully cleaned bones used in the investigation by Hartman and Meigs (*8*), the Ca/P ratio did not rise above 2.23:1 or fall below 2.14:1 for any single bone.

The effects of advancing age and of rations containing different amounts of calcium and phosphorus, on the relative amounts of various mineral elements in bones, have been studied by Neal, Palmer, Eckles, and Gullickson (*20*), and by Henderson and Weakley (*9*). The former group of investigators found that the ratio between calcium phosphate and calcium carbonate in bone decreases with advancing age and is also reduced by feeding rations low in phosphorus. The changes, though definite, are not very large, and would not produce any very great change in the ratio between the total calcium and the total phosphorus of the bone.

Henderson and Weakley (*9*) give figures for the total calcium and total phosphorus of the bones of cattle at various ages, that had been fed rations containing various amounts of calcium and phosphorus. Their results are in entire general agreement with those of Neal and his collaborators in showing that there is slightly more calcium in relation to phosphorus in the bones of older cattle and in those of cattle fed on rations low in phosphorus, but the differences are very small. Table 3 gives figures for the Ca/P ratios calculated from the data of Henderson and Weakley. These ratios do not fall below 2.06:1 nor rise above 2.20:1 in any case. The Ca/P ratio is obviously more affected by changes in age than by changes in feeding.

TABLE 3.—*The calcium-phosphorus ratio in the bones of cattle at various ages, that had been fed on rations containing various amounts of calcium and phosphorus, as calculated from the data of Henderson and Weakley* (*9*)

Character of ration	Ca/P ratio in bones of cattle, at—				Average
	8 months	13 months	19 months	25 months	
Normal	2.09	2.12	2.10	2.15	2.11
Low in calcium	2.06	2.10	2.11	2.14	2.10
Low in phosphorus	2.10	2.13	2.19	2.17	2.15
Low in both calcium and phosphorus	2.08	2.10	2.17	2.20	2.14
Average	2.08	2.11	2.14	2.16	

The studies made by Lawes and Gilbert (*14*) on cattle were much less extensive than those at the Missouri Station. As far as they go, however, they agree very closely with the Missouri results. Table 4 shows how close this agreement is for the calcium and phosphorus content of the fat-free bodies of cattle at different ages.

Lawes and Gilbert give no figures for the composition of the separated skeletons and soft tissues of their animals, but, by assuming that the soft tissues of their animals had the usual calcium content and that the bones had the usual proportional weight and Ca/P ratio, the phosphorus of the soft tissues can be estimated. The results of such calculations indicate that their animals had very nearly the same percentages of phosphorus in their fat-free soft tissues as the corresponding animals of the Missouri investigation.

TABLE 4.—*Average calcium, phosphorus, and fat in whole bodies and in fat-free bodies of cattle at about 3 months of age and at about 4 years of age, as indicated by 2 investigations*

Investigation	Calves, at about 3 months of age					Oxen, at about 4 years of age				
	Whole bodies			Fat-free bodies		Whole bodies			Fat-free bodies	
	Calcium	Phosphorus	Fat	Calcium	Phosphorus	Calcium	Phosphorus	Fat	Calcium	Phosphorus
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Lawes and Gilbert (*14*)	1.18	0.67	14.80	1.38	0.79	1.39	0.74	24.60	1.84	0.98
Missouri	1.34	.78	7.15	1.44	.84	1.36	.72	26.66	1.85	.98

There is a very satisfactory agreement, therefore, between the older British and the newer American investigations. From a study of the figures one gets the impression that the methods of determining calcium and phosphorus in the tissues of cattle were quite adequate as far back as 1859, and that Lawes and Gilbert were able to determine these elements with just about the same degree of accuracy as can be attained at the present time. One gets the impression also that the calcium and phosphorus content of the tissues of cattle varies only within narrow limits and according to certain definite laws.

This impression is strengthened by the fact that results very similar to those just discussed have been obtained in numerous investigations of the composition of the whole bodies of other species of animals, and of particular tissues of cattle as well as of other animals. It is not necessary to describe these at length, but it may be mentioned that Sherman (*24*, *25*) has shown that the proportional amounts of calcium and phosphorus in the bodies of rats increase with advancing age and that Katz (*13*) has obtained figures for the phosphorus content of ox and calf muscle which are very similar to those found in the Missouri investigations. Katz found 0.170 percent of phosphorus in ox muscle and 0.220 percent in calf muscle.

EXPERIMENTS IN GROUP 1

PRELIMINARY ESTIMATES OF CALCIUM ASSIMILATION IN LONG-CONTINUED FEEDING EXPERIMENTS FOLLOWED BY SLAUGHTER AND BONE ANALYSIS

The work reported in this section is a continuation and enlargement of work already published (*8*). The procedure employed has been fully described (*8*), and need only be outlined here. In the tabulated results the breed of all cows at the Beltsville Station is indicated by their numbers. Those numbered from 1 to 99 and from 300 to 399 are grade Jerseys; those numbered from 100 to 199, grade Holsteins; those numbered from 200 to 299, registered Holstein-Friesians; and those numbered from 400 to 499, registered Jerseys. The grain mixtures used are described in table 5.

TABLE 5.—*Composition of grain mixtures* [1] *used in the group 1 experiments*

Grain ration no.	Corn meal	Wheat bran	Cotton-seed meal	Soybean meal	Linseed meal	Salt
	Parts	*Parts*	*Parts*	*Parts*	*Parts*	*Parts*
50	40	30	20	----------	10	1
55	30	20	25	----------	25	1
60	40	30	----------	20	10	1
65	30	20	----------	25	25	1
75	40	40	----------	----------	20	1

[1] The corn meal used throughout the experiments was yellow. The oil meals had their fat extracted by the old process, that is, without fat solvents.

The calcium intake in the food of cows and the output through their milk and calves was determined for periods of from 1 to 6 years. At the end of these periods the cows either died or were slaughtered, and some of their bones were carefully cleaned, weighed, and analyzed. Some of the cows received liberal amounts of alfalfa hay in the experimental periods; others received only timothy hay or timothy hay and corn silage as roughage. From the figures for the calcium intake and output determined as above described, the percentage of food calcium utilized for the production of milk and calves can be calculated; the result has been called the "percentage calcium assimilation." This result, however, does not represent the true percentage of food calcium utilized, unless it can be assumed that calcium is neither gained nor lost by the body during the experimental period. In order to obtain an idea of how much calcium is likely to be lost by the body of a cow in a period of 2 or 3 years on timothy hay, studies of the bones of cows have been made as above outlined after periods on timothy and on alfalfa, respectively. These studies suggest that the losses on timothy are not likely to be large enough seriously to vitiate the determinations of percentage calcium assimilation in periods lasting more than 2 years, but the results obtained in the experiments under consideration are only approximate and leave many questions to be answered by the later complete slaughter analyses.

Tables 6 to 9 give a summary of the results already published, together with the results of new experiments to be described later.

TABLE 6.—*Ratio of bone weight to body weight in cows when fed alfalfa hay or timothy hay*

Cow no.	Ratio of bone weight to body weight in—	
	Long bones	Ribs
ALFALFA HAY		
17	0.00545	0.00191
84	.00555	.00186
90	.00576	.00168
N202	.00621	.00176
458	.00546	.00185
Average	.00569	.00181
TIMOTHY HAY		
N401	0.00589	
429	.00491	0.00155
499	.00525	.00155
Average	.00535	.00155

TABLE 7.—*Total amounts of feed and of calcium from the various feeds consumed by the cows when fed rations containing or lacking alfalfa hay*

RATIONS CONTAINING ALFALFA HAY

Cow no.	Experimental feeding period	Feed and calcium consumed							
		Grain		Alfalfa		Timothy		Silage	
		Total	Calcium	Total	Calcium	Total	Calcium	Total	Calcium
		Kilograms	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*
84	Jan. 1, 1927, to Oct. 28, 1932	[1] 10,758	12.6	9,158	124.9	1,616	5.1	8,225	8.2
N202 [2]	Jan. 1, 1927, to Aug. 20, 1929	[3] 5,113	6.6	4,892	62.2	------	------	------	------
458	Jan. 1, 1927, to Jan. 13, 1933	[4] 12,052	15.4	11,057	152.7	1,846	5.8	------	------

RATIONS CONTAINING NO LEGUME HAY

54	Nov. 1, 1924, to Jan. 31, 1927	[5] 4,899	7.9	------	------	2,852	8.2	5,335	5.2
N401	Apr. 1, 1929, to Dec. 24, 1931	[6] 6,457	13.3	------	------	4,651	17.9	------	------
429	Dec. 1, 1924, to May 31, 1927	[5] 5,828	9.3	------	------	3,283	9.6	5,819	5.7
450	Oct. 1, 1925, to Nov. 30, 1926	[5] 2,011	3.2	------	------	1,678	4.8	------	------
456	Aug. 1, 1924, to Mar. 31, 1927	[5] 4,767	7.6	------	------	3,240	9.2	4,972	4.9
499	Oct. 1, 1927, to Dec. 16, 1932	[6] 12,851	24.8	------	------	7,532	22.9	------	------

[1] 6,595 kg of grain 50, and 4,163 kg of grain 60.
[2] In addition to grain and hay, cow N202 consumed 3,576 kg of beets and 377 kg of beet pulp in the experimental period, containing 3 kg of calcium.
[3] Grain 60.
[4] 11,917 kg of grain 60, and 135 kg of grain 75.
[5] Grain 55.
[6] Grain 65.

TABLE 8.—*Intake and outgo of calcium and percentage of calcium assimilated by cows fed various rations*

Cow no.	Ration	Length of period	Total calcium in—			Calcium assimilated [1]	Total milk yield
			Food	Milk	Calves		
		Days	*Kilograms*	*Kilograms*	*Kilograms*	*Percent*	*Kilograms*
84	Grain, alfalfa, timothy, silage	2,128	150.8	17.47	1.67	12.7	13,929
N202	Grain, alfalfa, beets, beet pulp	963	68.8	7.69	1.33	13.1	6,866
458	Grain, alfalfa, timothy	2,205	173.9	28.79	1.39	17.4	18,599
54	Grain, timothy, silage	822	21.3	5.85	.36	29.2	5,851
N401	Grain, timothy	998	31.2	11.99	.85	41.2	8,046
429	Grain, timothy, silage	912	24.6	10.82	.61	46.5	8,260
450	Grain, timothy	427	8.0	2.42	.21	32.9	1,731
456	Grain, timothy, silage	973	21.7	6.45	.61	32.5	4,610
499	Grain, timothy	1,904	47.7	21.27	1.43	47.6	14,598

[1] Percentage of calcium assimilated $= \frac{\text{Ca in milk and calves}}{\text{Ca in food}} \times 100$.

TABLE 9.—*Composition of fresh clean bones of cows that had been fed rations containing alfalfa hay and rations containing no legume hay, respectively*

Cow no.	Ash		Calcium		Phosphorus		Protein [1]		Water		Ca/P ratio	
	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
GRAIN AND ALFALFA HAY												
17	48.6	51.4	18.8	19.8	8.7	9.1	20.3	24.9	18.5	17.7	2.16	2.18
90	46.8	47.1	18.1	18.2	8.4	8.4	20.6	23.7	11.7	16.0	2.15	2.17
N202	47.4	51.0	18.8	19.4	8.7	9.2	20.4	24.4	12.3	14.7	2.16	2.11
Average	47.6	49.8	18.6	19.1	8.6	8.9	20.4	24.3	14.2	16.1	2.16	2.15
GRAIN AND TIMOTHY HAY												
N401	46.5	43.1	17.8	16.4	8.5	7.8	20.7	23.6	12.6	22.0	2.09	2.10
429	47.9	46.2	18.7	18.2	8.6	8.2	22.0	25.2	12.2	18.7	2.17	2.22
Average	47.2	44.6	18.2	17.3	8.5	8.0	21.3	24.4	12.4	20.3	2.14	2.16

[1] N × 6.25.

The bones which were weighed and analyzed in the experiments under consideration were the radius and ulna, metacarpus, tibia, metatarsus, and the tenth, eleventh, twelfth, and thirteenth ribs from one side of the body. The ratios of bone weight to body weight in table 6 were obtained by dividing the sum of the weights of the long bones and the sum of the weights of the ribs respectively by the body weights of the cows. The figures for body weight were obtained as in the original investigation by averaging the monthly weights of the cows for periods of 2 to 3 years before their deaths, omitting the weights for 4 months before and 2 months after calving.

The figures in table 6 suggest that the loss of material from the long bones of cows fed for long periods on timothy hay is very small. The loss from the ribs appears to be larger, but the results are too few to justify any positive conclusions. For this reason, the figures for calcium assimilation in table 8 have been calculated without making any allowance for possible loss of bone material during the experimental periods. This matter will be discussed after the results of the slaughter experiments have been given. The figures, as they stand, suggest that cows on timothy rations are likely to assimilate much larger proportions of the calcium intake than those on alfalfa rations.

Table 9 gives the composition of the carefully cleaned bones of several cows which had been fed alfalfa and timothy rations, respectively, for long periods. The results show the strong tendency toward constancy in bone composition, and particularly the constancy of the Ca/P ratio. The results also suggest that the ribs are affected more than the long bones by rations low in calcium. The figures in table 9 show that the percentages of ash, calcium, and phosphorus were only slightly lower in the long bones of the cows fed timothy than in those of the cows fed alfalfa, whereas these percentages in the ribs of the cows fed timothy were considerably lower.

While these somewhat fragmentary studies of the bones of cattle that have been fed different rations for long periods suggest that long

periods on rations low in calcium have only a rather small effect on the weight and composition of the bones, such studies are obviously much less satisfactory than would be the determination of the total quantities of calcium, phosphorus, fat, and other materials in the whole bodies of cattle under such circumstances. The results of such determinations are given in the section following.

EXPERIMENTS IN GROUP 2

BELTSVILLE SLAUGHTER EXPERIMENT

The three cows studied were nos. 84, 458, and 499. These cows were fed fairly uniform rations without pasture for approximately 6 years before they were slaughtered. The percentage of calcium assimilated during the experimental periods was determined according to methods previously outlined (*8*). The results along with those of other cows subjected to somewhat similar treatment are given in table 8. Only a few details need be added here.

Cow 84, born October 11, 1917, was started on the experiment January 1, 1927, on a moderately high-calcium ration, receiving grain 50, alfalfa hay, and corn silage until April 1931. From then to August 1931, she received several different rations containing grain 50, grain 60, alfalfa hay, timothy hay, and corn silage. From August 1931 to the date of slaughter, she received grain 60, alfalfa hay, and timothy hay. Her average daily calcium intake was 70.9 g. Her last calf was born October 15, 1932; she was slaughtered October 28, 1932, and weighed 399.6 kg just before slaughter.

Cow 458, born June 20, 1921, was started on the experiment January 1, 1927, on a high-calcium ration, receiving grain 60 and alfalfa hay through May 1931. From then until September 1932 she received grain 60 with alfalfa and timothy; and from then until the date of slaughter she received grain 60 with alfalfa hay alone. Her average daily calcium intake was 78.9 g. Her last calf was born December 6, 1932; she was slaughtered January 13, 1933, and weighed 394.1 kg just before slaughter.

Cow 499, born April 29, 1923, was started on the experiment October 1, 1927, on a low-calcium ration, receiving grain 65 and timothy hay until the date of slaughter. Her average daily calcium intake was 25.1 g. Her last calf was born November 19, 1932; she was slaughtered December 16, 1932, and weighed 432.6 kg just before slaughter.

All hay fed to these three cows was U. S. No. 1 grade (*21*). The calcium content of the grain and silage fed was calculated by multiplying the total consumption by average figures for the calcium content of grain and silage, obtained from a number of analyses of samples of these feeds used at the Beltsville Station. Each batch of hay bought was analyzed, and the calcium content of the amount of this batch consumed by each cow was calculated from this analysis. It was thought better to follow this more laborious procedure with the hay on account of the greater variability in its composition.

The milk of each of the three cows was analyzed a number of times, and the averages of these analyses for each cow were used in calculating the calcium content of her milk. Table 10 gives some of the details regarding these analyses, and also a comparison of some of the Beltsville figures for the feeds with the average figures given by Henry and Morrison (*10*).

TABLE 10.—*Data regarding the calcium analyses of the feed and milk of cows 84, 458, and 499 in the experimental periods previous to slaughter*

Material analyzed	Analyses	Calcium found in Beltsville analyses			Average calcium figures of Henry and Morrison [1]
		Maximum	Minimum	Average	
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	27	2.406	0.962	1.424	1.393
Timothy hay	20	.564	.175	.322	.179
Grain 50	5	.129	.093	.111	.112
Grain 60	35	.157	.102	.128	
Grain 65	18	.233	.171	.193	
Corn silage	4	.104	.093	.100	
Milk from—					
Cow 84	5	.131	.114	.125	
Cow 458	5	.158	.148	.155	
Cow 499	14	.157	.134	.146	

[1] Henry and Morrison (*10*) give no figures for soybean meal from which the fat has been extracted, or for corn silage.

The animals were slaughtered in the usual manner by a blow on the head followed by bleeding. The blood was collected in a tub and weighed. To determine its composition samples were obtained shortly before slaughter and analyzed.

After the animals had been drained of blood, the hides were removed and weighed. Representative samples were then cut from them as described by Small (*26*, *27*). These were cut with safety-razor blades into small pieces, from which samples were taken and analyzed.

From the skinned carcasses the heads, tails, lower leg joints, and abdominal and thoracic organs were removed. The remainder was sawed very carefully into two equal halves down the middle of the back bone. Each of these halves was weighed and placed in the cooler.

The intestinal tract was tied off at each end before it was removed from the body. After it was removed, it was weighed together with its contents. The contents were then washed out, the empty tract weighed, and the weight of the contents obtained by the difference.

Of the organs, the liver, kidneys, and intestinal tract were weighed and analyzed separately. All the other organs and the nervous system were weighed, ground up, and analyzed as one sample, called "miscellaneous organs" in the tables.

Three samples of meat were weighed and analyzed separately; namely, the ninth, tenth, and eleventh rib cut, minus the so-called "eye" from this cut, the "eye", and the round steak from the hind quarter. The greater part of the rest of the meat and fat from one side of the carcass was weighed and analyzed as one sample. Before the carcass was cut up, however, the kidney and bed fat were removed and weighed as a separate sample.

The meat was roughly separated from the bones of the head, tail, and lower leg joints and put into the sample called "miscellaneous meat and fat." The same procedure was followed with the bones from the carcass. The radius and ulna, tibia, metacarpus, metatarsus, and the tenth, eleventh, twelfth, and thirteenth ribs of the carcass bones were carefully cleaned and weighed in the green state. Their weights were compared with the body weights; the results of the com-

parison are given in table 6. The other bones of the carcass were only roughly cleaned with a knife. All the roughly cleaned bones were boiled for some time, after which most of the adhering meat, fat, tendon, etc., was easily removed. This material was added to the "miscellaneous meat and fat", while the bones thus cleaned were united with the carefully cleaned bones, and the whole sample was ground and analyzed. This is the portion called "bones" in table 11.

The hoofs were removed from the lower leg by heating in boiling water, and separately analyzed.

The percentage composition of the parts into which the cows were divided and the percentage composition of the live empty weight and of the fat-free empty weight are given in table 11. The following description will show how these figures were obtained and how they are to be interpreted.

The cows were given no food for 24 hours before slaughter. Each was weighed just before slaughter (p. 10). The live empty weight is obtained by subtracting the weight of the intestinal contents from the live weight just before slaughter. The fat-free empty weight is obtained by subtracting the weight of the total fat contained in the body from the live empty weight.

The figures for the weights of the various parts of the body—bones, hide, and hair, etc.—give the weights of the parts as obtained just before samples were taken out for analysis, except that, in the case of parts taken from one side of the body only, as kidney, carcass meat, etc., these weights are multiplied by two. The figures for the percentage composition of the parts give the composition of the parts as analyzed. The true weight of each constitutent—calcium, phosphorus, etc.—of each part is, therefore, obtained by multiplying the weight of the part by the percentage figure and dividing by 100. The percentage of each constitutent in the live empty weight and in the fat-free empty weight is obtained by dividing the sum of the weights of that constituent in all the parts by the live empty weight, etc., and multiplying by 100. The figures for the composition of the live empty weight and of the fat-free empty weight, therefore, give the true percentage composition of these parts as constituents of the animal just before slaughter.

The figures for the composition of all of the other parts, except the miscellaneous meat and fat and possibly the bones, are slightly higher than they would be in the live animal, because these parts lost some water in the processes of cooling and mincing or grinding for analysis. It was determined that the carcass lost about 3 percent of its weight during cooling. The situation is altered in the case of the miscellaneous meat and fat and possibly the bones by the fact that the roughly cleaned bones were boiled and allowed to cool under water, and that the soft tissues thus removed from the bones were placed in the portion called miscellaneous meat and fat. The soft tissues adhering to the bones probably took up water during this process, and the figures for the composition of the miscellaneous meat and fat are, therefore, probably lower than they would be in the live animal.

The sum of the weights of all the parts of cow 84 is about 14 kg lower than the live weight just before slaughter, while of the other two cows the live weights are very nearly equal to the sums of the weights of the parts. It seems probable that this is due to the fact that more care was taken to remove the soft tissues from the bones

of cow 84 by means of the knife than in the other two cases. In her case, therefore, the sample called miscellaneous meat and fat is much smaller than in the other two cases, and the taking up of water by the portion of this sample that came from the roughly cleaned bones does not come so near to compensating for the loss of water by the other tissues during cooling.

TABLE 11.—*Weight and percentage composition of body parts into which cows 84, 458, and 499 were divided for study*

COW 84

Body or body part considered	Weight	Calcium	Phosphorus	Ash	Nitrogen	Water	Fat
	Kilograms	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Live empty weight	337.3	1.76	0.93	5.24	3.26	62.91	7.08
Fat-free empty weight	313.4	1.90	1.00	5.64	3.51	67.71	
Intestinal contents	62.3						
Bones	36.2	16.21	7.47	41.71	3.28	27.05	9.48
Hoofs	.7	.083	.055	3.98	10.42	34.04	.21
Hide and hair	27.2	.014	.037	.55	5.72	65.75	1.02
Kidney and bed fat	2.6	.078	.174	.73	1.86	37.38	50.25
Liver	7.5	.008	.311	.90	3.32	74.08	2.95
Kidneys	1.4	.013	.214	.90	2.53	79.12	2.90
Intestinal tract	26.3	.022	.134	.70	2.31	79.72	5.02
Blood	24.5	.0067	.0195	.92	2.61	82.36	.00
Miscellaneous organs	38.3	.060	.166	.60	2.26	70.92	14.00
Carcass meat	133.9	.013	.182	.95	3.51	70.98	5.60
Rib cut minus eye	3.2	.012	.174	.70	3.39	68.40	8.74
Eye from rib cut	1.5	.0055	.205	1.00	3.60	76.32	1.02
Round steak	6.3	.0044	.215		3.61	73.90	2.51
Miscellaneous meat and fat	13.6	.144	.234	2.12	4.77	39.96	29.15

COW 458

Live empty weight	343.4	1.62	0.85	4.79	2.85	57.07	20.27
Fat-free empty weight	273.8	2.04	1.07	6.01	3.57	71.58	
Intestinal contents	50.7						
Bones	33.9	16.22	7.50	41.56	3.15	24.80	10.83
Hoofs	.9	.073	.043	.64	11.20	34.66	.087
Hide and hair	26.5	.022	.052	.83	6.19	62.99	1.04
Kidney and bed fat	10.6	.0018	.0076	.13	.39	9.60	87.12
Liver	6.2	.0060	.359	1.37	3.49	71.25	4.46
Kidneys	1.3	.0074	.220	1.09	2.38	79.89	2.86
Intestinal tract	29.1	.031	.118	.66	1.76	77.05	10.08
Blood	21.3	.0067	.0175	.93	2.96	79.78	.00
Miscellaneous organs	44.2	.024	.060	.65	1.50	47.48	43.14
Carcass meat	121.4	.0086	.155	.74	2.92	61.50	19.11
Rib cut minus eye	3.4	.016	.097	.65	2.32	47.62	30.62
Eye from rib cut	1.1	.0052	.220	1.13	3.60	71.06	4.82
Round steak	3.0	.0048	.219	1.05	3.48	72.70	2.90
Miscellaneous meat and fat	41.3	.102	.132	.92	2.70	59.15	23.58

COW 499

Live empty weight	389.2	1.25	0.70	3.77	2.50	52.36	29.29
Fat-free empty weight	275.2	1.74	.97	5.26	3.49	73.03	
Intestinal contents	43.4						
Bones	33.4	14.12	6.89	36.69	2.90	27.10	14.90
Hoofs	.9	.063	.036	.52	10.64	34.20	.09
Hide and hair	29.0	.016	.036	.81	5.55	67.14	1.24
Kidney and bed fat	9.1	.0077	.020	.14	.37	5.67	92.46
Liver	5.4	.0045	.372	1.34	3.49	66.96	8.71
Kidneys	1.1	.0098	.194	1.12	2.35	78.00	4.08
Intestinal tract	26.0	.013	.096	.59	1.94	77.82	11.62
Blood	21.1	.0052	.0202	.95	2.98	79.70	.00
Miscellaneous organs	52.4	.020	.083	.44	1.10	34.92	55.14
Carcass meat	167.5	.0068	.118	.61	2.37	48.20	36.94
Rib cut minus eye	4.8	.0068	.076	.38	1.70	32.56	56.60
Eye from rib cut	1.5	.0052	.205	1.00	3.52	68.60	8.25
Round steak	4.6	.0044	.208	1.03	3.61	72.48	3.46
Miscellaneous meat and fat	32.4	.094	.115	.62	2.22	77.82	8.95

The results of the analyses in table 11 will be discussed further in connection with the Missouri results, in the general discussion.

EXPERIMENTS IN GROUP 3

BALANCE EXPERIMENTS WITH COWS THAT HAD BEEN FED TIMOTHY RATIONS FOR LONG PERIODS

In work carried out at the Wisconsin and Beltsville Experiment Stations, and reviewed a few years ago (*15*), the calcium assimilation of cows fed alfalfa and timothy hay ranged from 5 to 28 percent of the intake. The percentage assimilation when fed timothy hay showed no tendency to be higher than when fed alfalfa hay. In the work of Huffman (*12*) and Ellenberger (*4*), on the other hand, the calcium assimilation of cows fed timothy hay was often about 50 percent of the intake.

In the Beltsville work, and probably also in the Wisconsin work, the cows had been fed rations containing alfalfa hay and liberal quantities of calcium for long periods before the calcium balances were determined. In Huffman's experiments, on the other hand, and probably also in Ellenberger's, the cows had been fed low-calcium rations for long periods.

In view of the work described in the foregoing part of this paper, it seems probable that these different tendencies to assimilate calcium were due to the fact that the cows which had been fed alfalfa still had a considerable surplus stock of calcium in their bones when the balances were determined, while those which had been fed timothy had reached the bottom of their reserves, or nearly so. But, for a number of reasons, it has seemed worth while to carry out some further experiments on this subject. These reasons may be stated as follows: Work at the Wisconsin (*6*) and Beltsville (*15*) Stations indicates that cows which have previously been fed rations high in calcium may continue for considerable periods to lose calcium from their bodies when transferred to rations low in calcium. The cow used in the Wisconsin work apparently lost calcium from her body continuously during a period of 16 weeks in which the balance was determined at intervals. Two cows used in the Beltsville work lost calcium continuously for a period of 19 weeks.

This long-lasting influence of previous treatment may explain many of the apparently discordant results of balance experiments, in most of which little is said about the previous treatment of the animals. It seems justifiable, therefore, to put on record the results of a balance experiment at Beltsville in which this point is taken into account. Three cows were used in the experiment—N105, N216, and N406. Data regarding their history previous to the beginning of the balance experiment are given in table 12. As may be seen from the table, all the cows had been fed low-calcium rations for more than 8 months before the balance experiment started.

TABLE 12.—*Historical record of the three cows used in the balance experiments at Beltsville, Md.*

Cow no.	Date of birth	Put on low-calcium ration	Constituents of ration	Last calving before balance experiment	Balance experiment started
N105	Aug. 5, 1927	July 1, 1930	Grain 65, no. 1 timothy hay, corn silage.	May 23, 1931	Mar. 2, 1932
N216	Jan. 26, 1929	Apr. 1, 1931	Grain 65, no. 1 timothy hay	June 21, 1931	Do.
N406	Oct. 1, 1928	June 4, 1931	do	Oct. 18, 1931	Do.

The data pertaining to the balance experiment are given in tables 13 to 16. The balance experiment proper began March 2, 1932. From this time on the rations for all these cows consisted of grain 65 and no. 1 timothy hay, coarsely chopped. As in previous experiments at this station, the output of calcium and phosphorus in the feces and urine of each weekly period is balanced against the intake in the food for a weekly period beginning 2 days earlier, in order to allow for the intestinal lag.

TABLE 13.—*Water, calcium, and phosphorus content of feeds used at different times in the balance experiments*

Feed	Period of feeding	Water	Calcium	Phosphorus
		Percent	*Percent*	*Percent*
Timothy hay (T 35)	Mar. 2 to 29	10.50	0.280	0.171
Grain 65 (batch 1)	Mar. 2 to 12	11.92	.165	.684
Grain 65 (batch 2)	Mar. 13 to 22	12.16	.166	.663
Grain 65 (batch 3)	Mar. 23 to 29	11.56	.179	.644

TABLE 14.—*Calcium and phosphorus content of the milk from 3 cows in the balance experiments*

Experimental period	Cow N105		Cow N216		Cow N406	
	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Mar. 4 to 10	0.092	0.097	0.119	0.098	0.148	0.136
Mar. 11 to 17	.091	.096	1.22	.101	.149	1.36
Mar. 18 to 24	.093	.096	.135	.098	.154	.134
Mar. 25 to 31	.094	.095	.120	.106	.150	.132

TABLE 15.—*Average daily feed consumption by 3 cows in the balance experiments*

Experimental period	Cow N105		Cow N216		Cow N406	
	Grain	Hay	Grain	Hay	Grain	Hay
	Kilograms	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*	*Kilograms*
Mar. 2 to 8	6.60	7.43	6.58	5.11	[1] 6.60	[1] 5.50
Mar. 9 to 15	6.60	8.00	7.00	5.50	6.60	6.00
Mar. 16 to 22	6.60	8.00	5.50	3.93	6.60	6.00
Mar. 23 to 29	6.60	8.00	4.00	3.93	6.60	6.00

[1] Mar. 3 to 8; see text.

One batch of hay (T35) was sufficient for the entire experiment. Three batches of grain were used.

The cows were fed hay and grain twice a day, and were milked three times a day. They had water at will. They were exercised 10 to 15 minutes daily except Sunday.

A mistake was made in taking the aliquot of the first day's feces of cow N406, so the first period for this cow consists of only 6 days. Cow N216 was off feed and showed a reduced milk yield in the third and fourth periods. The 4.81 kg of refused feed taken from her manger during these periods contained 0.393 percent of calcium and 0.597 percent of phosphorus. Her appetite improved and her milk yield increased toward the end of the fourth period. Except for these incidents, the experiment ran smoothly.

TABLE 16.—*Average daily milk yield, and calcium and phosphorus intake, output, and balance of the 3 cows in the balance experiment*

Experimental period	Milk yield	Calcium							Phosphorus							Ca/P ratio in feed
		Output			Total intake	Balance	Assimilation		Output			Total intake	Balance	Assimilation		
		In urine and feces	In milk	Total					In urine and feces	In milk	Total					
	Kg.	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	
COW N105																
First week	11.8	23.8	10.8	34.6	31.7	−2.9	7.9	25.0	48.8	11.4	60.2	57.8	−2.4	9.1	15.7	0.55
Second week	11.9	24.3	10.8	35.1	33.3	−1.8	9.0	27.0	48.7	11.4	60.1	58.1	−2.0	9.5	16.3	.57
Third week	11.7	25.7	10.9	36.6	33.4	−3.2	7.7	23.1	47.4	11.3	58.7	57.4	−1.3	9.9	17.3	.56
Fourth week	11.4	28.9	10.7	39.6	34.2	−5.4	5.3	15.5	47.3	10.8	58.1	56.2	−1.9	8.9	15.8	.61
Average	11.7	25.7	10.8	36.5	33.3	−3.2	7.5	[1] 22.5	48.1	11.2	59.3	57.4	−1.9	9.3	[1] 16.3	.57
COW N216																
First week	11.7	14.3	13.9	28.2	25.0	−3.2	10.7	42.8	48.5	11.5	60.0	53.2	−6.8	4.6	8.7	0.47
Second week	12.3	14.7	15.0	29.7	27.0	−2.7	12.3	45.5	46.1	12.4	58.5	56.5	−2.0	10.4	18.4	.48
Third week	7.7	9.0	10.5	19.5	17.4	−2.1	8.4	48.3	31.0	7.6	38.6	39.1	+.5	8.1	20.8	.45
Fourth week	8.5	11.7	10.2	21.9	18.2	−3.7	6.5	35.7	25.7	9.0	34.7	32.5	−2.2	6.7	20.7	.56
Average	10.1	12.4	12.4	24.8	21.9	−2.9	9.5	[1] 43.3	37.8	10.1	47.9	45.3	−2.6	7.5	[1] 16.4	.49
COW N406																
First week	9.6	15.3	14.2	29.5	26.3	−3.2	11.0	41.9	43.6	13.0	56.6	54.5	−2.1	10.9	20.0	0.48
Second week	9.5	15.0	14.1	29.1	27.7	−1.4	12.6	45.5	42.7	12.9	55.6	54.7	−.9	12.0	21.9	.51
Third week	9.6	16.7	14.8	31.5	27.8	−3.7	11.1	39.9	42.2	12.9	55.1	53.9	−1.2	11.7	21.7	.52
Fourth week	9.6	17.2	14.4	31.6	28.6	−3.0	11.4	39.8	41.3	12.6	53.9	52.8	−1.1	11.5	21.8	.54
Average	9.6	16.0	14.4	30.4	27.6	−2.8	11.6	[1] 41.8	42.5	12.9	55.4	54.0	−1.4	11.5	[1] 21.3	.51

[1] Based on totals.

As may be seen from table 16 the average calcium assimilation of cows N216 and N406 was between 40 and 50 percent of the intake. There seems every reason to believe that cows may readily assimilate from 40 to 60 percent of the calcium intake when they are kept on low-calcium rations for long periods. The calcium balances were negative throughout, though not very markedly so.

GENERAL DISCUSSION

COMPARISON OF WORK AT BELTSVILLE WITH THAT IN MISSOURI

The figures in table 11 for the composition of the whole bodies of the cows and of the various portions into which their bodies were divided may be compared with those given in Missouri Research Bulletin 55 (*18, tables 1 to 30*). The two sets of figures agree in many respects, and further study shows that much of the apparent disagreement is due to the varying fat content in the whole bodies and portions of the different animals used. It is easy, of course, to calculate the percentages of calcium, phosphorus, ash, etc., in the fat-free bodies and portions of the animals used; and this has been done to some extent in order to obtain the figures shown in tables 17 and 18.

TABLE 17.—*Calcium and phosphorus in the fat-free bodies of cattle and in various fat-free portions of their bodies, as calculated from analyses made at the Beltsville and Missouri Stations*

BELTSVILLE RESULTS

Kind of animal and no.	Whole body		Total soft tissues		Carcass meat		Bones		Blood		Hide and hair	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Cow 84	1.90	1.00	0.031	0.153	0.014	0.193	17.91	8.25	0.0067	0.0195	0.014	0.037
Cow 458	2.04	1.07	.038	.147	.011	.192	18.19	8.51	.0067	.0175	.022	.053
Cow 499	1.74	.97	.029	.149	.011	.187	16.59	8.10	.0052	.0202	.016	.036
Average	1.89	1.01	.033	.150	.012	.191	17.56	8.29	.0062	.0191	.017	.042

MISSOURI RESULTS [1]

Calves	1.45	0.84	0.022	0.186	0.013	0.207	6.61	3.23	0.0070	0.0214	0.054	0.099
Oxen	1.84	.98	.017	.143	.014	.169	11.81	5.55	.0065	.0160	.033	.047
Cows	------	1.04	------	.197	------	.212	------	6.86	------	.0273	------	.064

[1] The Missouri figures for calves and oxen are calculated from the figures given in Research Bulletin 107 (*11, tables 6 to 28*). Those for calves are averages for the animals 8 months old and younger; those for oxen, averages for the animals 40 months old and older. The Missouri figures for cows are calculated from those given in Research Bulletin 61 (*19*) and are averages for the 3 cows used.

In table 17 the Beltsville figures for the calcium and phosphorus content of the fat-free bodies and of various portions of the body are compared with corresponding figures calculated from the Missouri data. The figures for the whole body and for the carcass meat [4] are in close agreement with the corresponding Missouri figures for mature oxen and cows. It has already been shown that the Missouri figures agree with the figures of Lawes and Gilbert (*14*) for the calcium and phosphorus content of the fat-free bodies of mature cattle, and it may therefore be assumed that these quantities are nearly constant. As the Beltsville figures agree with those established by the two other investigations, it may be supposed that the analyses are satisfactorily accurate.

TABLE 18.—*Calcium and phosphorus content of live empty weights of cows and of all portions of their bodies calculated on the fat-free basis*

Body part	Calcium			Phosphorus		
	Cow 84	Cow 458	Cow 499	Cow 84	Cow 458	Cow 499
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Live empty weight	1.90	2.04	1.74	1.00	1.07	0.97
Total soft tissues	.031	.038	.029	.153	.147	.149
Bones	17.91	18.19	16.59	8.25	8.51	8.10
Hoofs	.083	.074	.064	.055	.043	.036
Hide and hair	.014	.022	.016	.037	.053	.036
Kidney and bed fat	.157	.014	.102	.350	.059	.265
Liver	.008	.006	.005	.324	.376	.407
Kidney	.013	.008	.010	.220	.226	.202
Intestinal tract	.023	.034	.015	.141	.131	.109
Blood	.0067	.0067	.0052	.019	.017	.020
Miscellaneous organs	.070	.042	.045	.193	.106	.185
Carcass meat	.014	.011	.011	.193	.192	.187
Rib cut minus eye	.013	.023	.016	.191	.140	.175
Eye from rib cut	.0056	.0055	.0057	.207	.231	.223
Round steak	.0045	.0049	.0046	.221	.226	.215
Miscellaneous meat and fat	.203	.133	.103	.330	.173	.126

There is some disagreement between the Beltsville figures and the Missouri figures for other portions of the body, particularly the bones. The Beltsville figures would seem to indicate that bones have percentages of calcium and phosphorus about 50 percent higher than those shown by the Missouri figures. This discrepancy is caused by the different methods by which the portion called skeleton or bones was prepared in the two investigations. In the Beltsville work some of the long bones and ribs were very carefully cleaned before being ground for analysis, and the rest of the skeleton was boiled and then stripped of all adhering soft tissue. In the Missouri work, on the other hand, the skeleton was only roughly cleaned by means of the knife, and the sample so prepared, consisting of bone with a good deal of adhering tendon and other soft tissue was ground and analyzed. It may be pointed out that in the Missouri work the portion called skeleton or bones comprised about 50 percent more of the weight of the total fat-free body than in the Beltsville work, and also that the Beltsville figures for the calcium and phosphorus content of the total bones of cows 84, 458, and 499 agree fairly closely with corresponding figures for the carefully cleaned bones of a number of cows given in table 9. Furthermore, the Beltsville figures agree with those given for clean bones by Henderson and Weakley (*9*).

The difference in procedure in the Beltsville and Missouri investigations, which has just been considered, explains some of the other discrepancies which appear in table 17. In the Beltsville investigation the scrapings from the carefully cleaned bones and the material removed by boiling from the rest of the skeleton were included in the portion called miscellaneous meat and fat. This portion has, therefore, a considerably higher calcium content than any of the other soft tissues. As the miscellaneous meat and fat is included in calculating the composition of the total soft tissues this latter portion has also a rather high calcium content.

[4] The portion described as "carcass meat" in the Beltsville tabulations corresponds to that called "lean and fat" in the Missouri bulletins.

The Beltsville figures for the calcium content of blood are in fairly close agreement with the Missouri figures. For the phosphorus content of the blood, the Beltsville figure lies between the Missouri figure given for oxen (*11*) and that given for cows (*19*). This latter figure is certainly unusually high, but it is well known that the phosphorus content of blood is quite variable. The Beltsville figure for the calcium content of hide and hair is decidedly lower than the Missouri figure, but it seems not unlikely that the small calcium content of this tissue may be subject to variations of this order.

TABLE 19.—*Calcium and phosphorus content and the Ca/P ratio for the live-empty weight of individual mature steers and cows calculated on a fat-free basis*

STEERS [1]

Manner of feeding and animal no.	Age at slaughter	Fat-free bodies		
		Calcium	Phosphorus	Ca/P ratio
High fed:	*Months*	*Percent*	*Percent*	
527	40	1.72	0.93	1.85
513	44	1.75	.94	1.86
501	48	1.78	.95	1.87
Medium fed:				
526	40	1.84	.99	1.86
502	44	1.79	.95	1.88
512	48	2.07	1.08	1.91
Low fed:				
524	40	1.96	1.04	1.88
509	44	1.75	.93	1.89
500	48	1.87	.99	1.89
Average		1.84	.98	1.88

COWS [2]

Fed for maintenance: 63	71		1.13	
Fattened:				
4	112		1.03	
43	85		.96	
Average			1.04	

[1] The figures for steers are calculated from those given in Bulletin 55 (*18, tables 69 to 71*) and in Bulletin 107 (*11, table 23*).
[2] The figures for cows are taken from Bulletin 61 (*19, table 9.*)

In table 19 are given the Missouri figures for the calcium and phosphorus content of the fat-free live empty weights of individual mature oxen and cows, which show the ranges of variation and the averages for these quantities under feeding conditions where all the animals had fairly liberal amounts of calcium in their rations. These figures are to be compared with those for the calcium and phosphorus content of the fat-free bodies of the Beltsville cows given in table 18. It will be seen at once that the differences between the calcium and phosphorus content of the Beltsville cows, 458 and 499, lie within the range of variation for the Missouri animals. If there were no other evidence on the subject than the figures given in these two tables, the results might be taken to show that the quantity of calcium contained n the food of a cow over a long period has little or no influence on the calcium and phosphorus content of her body.

It does not seem, however, that this is the most probable interpretation of the results. Cows 458 and 499 were of similar breed and type and of about the same age, and the weights of their fat-free bodies

and of their bones at slaughter were very nearly equal. Further, the results of balance experiments have repeatedly shown that cows may lose considerable amounts of calcium and phosphorus from their bodies and that these losses are likely to be larger when the calcium content of the ration is low.

Finally, the calcium and phosphorus content of cow 458 is near the upper range of the variation in the Missouri work, while that of cow 499 is near the lower range. The more probable interpretation of the results would seem to be that the two cows had about the same calcium and phosphorus content at the beginning of the experimental feeding periods, and that cow 499 lost about 15 percent of her calcium and about 10 percent of her phosphorus in the period during which she was fed timothy hay.

If this interpretation may be accepted as correct, the results suggest that it may be somewhat disadvantageous to feed cows as little calcium as was contained in the ration of cow 499. The fact, that in the same part of the lactation cycle she had 15 percent less calcium in her body than cow 458, might be taken to indicate that her ability to assimilate calcium from her food was put under a slight strain. In the work of Reed and Huffman (*22*), already referred to, the cows that got bone meal in addition to a basal ration of grain, timothy hay, and corn silage, seemed to do a little better than those that got the basal ration alone, and this gives some further basis for the views outlined above.

In regard to the question of the percentage of calcium assimilated, it makes little difference whether or not it be supposed that cow 499 lost 15 percent of the calcium content of her body in the 5-year experimental period preceding her slaughter. As shown in table 8, her calcium assimilation in this period, on the assumption that she lost no calcium from her body, was 47.6 percent of the intake. If it is assumed that 15 percent of her body calcium must be subtracted from that part of the output in her milk and calves which she was obliged to supply from her food, her calcium assimilation would be 45.8 percent. This result strongly confirms the conclusion to be drawn from the rather incomplete experiments described in the early part of this paper and from the balance experiments of Huffman (*12*) and Ellenberger (*4*), namely, that cows which are kept for long periods on rations low in calcium may readily assimilate from 40 to 50 percent of the calcium intake.

Table 18 gives the calcium and phosphorus content of the various portions of the three Beltsville cows calculated on the fat-free basis. Most of the individual tissues of cow 499 show a somewhat lower calcium content than those of cows 84 and 458, which adds strength to the supposition stated above that her low-calcium ration caused some loss of calcium from her body. In most cases, however, the differences are small, and the constancy of the calcium content of some of the portions, namely, the kidneys and muscles, is quite remarkable. The phosphorus content also of most of the soft tissues is remarkably constant.

Table 18 gives an idea of how calcium and phosphorus are lost from the body of a cow on a low-calcium ration. Much the greater portion of the loss comes from the bones, and the low-calcium ration causes so much loss of phosphorus from the bones that the Ca/P ratio is only slightly lower after the losses have occurred than before.

There is no evidence of any general tendency for phosphorus to be stored in the soft tissues, and both the calcium and phosphorus contents of the soft tissues is so small and so constant that the change in the Ca/P ratio in the whole body is almost entirely controlled by that which occurs in the bones. The Ca/P ratios in the bones of cows 84, 458, and 499 were 2.17, 2.14, and 2.05, respectively; those in their fat-free empty weights, 1.90, 1.91, and 1.79. These figures are to be compared with the extremely constant Ca/P ratios given in table 19 for the oxen analyzed at the Missouri Station. They constitute additional evidence for the view that the low-calcium ration of cow 499 had an effect on the calcium and phosphorus content of her body.

The results which have just been discussed confirm results obtained at the Michigan Station (*12*) and at the Vermont Station (*4*) which indicate that cows kept on low-calcium rations for long periods may readily assimilate about 50 percent of the calcium intake. They furnish strong reason for believing, however, that those aspects of the Vermont work which indicate that mature milking cows kept on low-calcium rations for long periods may store large quantities of calcium and phosphorus in proportions very different from those ordinarily found in the body, are due to experimental error. They also give reason to believe that the results of many other balance experiments which suggest that milking cows on high-calcium rations are usually losing calcium from their bodies are due either to experimental error or to some peculiarity in the experimental method which renders the results not representative of the usual course of calcium and phosphorus metabolism in long periods of time. The Beltsville results suggest that even on rations low in calcium it is difficult to reduce the calcium content of a cow's body to less than 85 percent of the normal level. In view of the high degree of constancy of the percentages of calcium and phosphorus in the mature fat-free bovine body, and of a number of difficulties in the technic of balance experiments, the method of slaughter and body analysis seems preferable to the balance method, when calcium and phosphorus metabolism is to be studied over long periods.

PRACTICAL CONSIDERATIONS

The practical object of such work as that just described is, of course, to determine the requirements of dairy cows for various nutritional materials, and the pathological effects which are produced by deficiencies of such materials. In 1924, when the experiments were started, there was much evidence on hand to show that feeding low-calcium roughage, such as wheat straw and timothy hay, to dairy cows was likely to cause premature calving and the throwing of dead calves; and many investigators were inclined to attribute these results to the low-calcium content of the roughage. With the passage of time, however, it has become clear that timothy hay and wheat straw are likely to be deficient not only in calcium, but also in vitamin A; and the relative nutritional qualities of these roughages and of alfalfa hay cannot be intelligently discussed without taking this matter into consideration.

Recent work at the writers' station has thrown some light on the vitamin A content of alfalfa and timothy hay. The results may be outlined as follows: The vitamin A content of hay is extremely

variable. There is no doubt, however, that it tends to be higher in alfalfa than in timothy hay, and higher in hay of good quality than in hay of poor quality. It is still too early to give figures which can be regarded as reliable averages, but the results so far obtained may be tentatively taken to indicate that No. 1 alfalfa hay contains about 40 international units of vitamin A activity per gram; No. 3 alfalfa, about 6 units; No. 1 timothy, about 15 units; and No. 3 timothy, about 3 units.

In still other unpublished experiments at the writers' station, cows have been fed for periods of several years on rations of grain combined with each of the above-mentioned kinds of hay as the sole roughage. Of these cows, only those fed on No. 1 alfalfa have reproduced satisfactorily. Those fed on No. 3 alfalfa and on No. 3 timothy have uniformly calved prematurely and thrown weak or dead calves. Those fed on No. 1 timothy have usually thrown weak or dead calves prematurely, but cow 499 has had four normal calves in a period of 5 years on this ration.

The writers have made numerous determinations of the calcium content of the No. 3 alfalfa hay, and find that it is practically the same as that of the No. 1 alfalfa. The results obtained at this station, therefore furnish clear evidence for the view that cows will calve prematurely and will throw dead or weak calves when their rations are deficient in vitamin A, even though such rations may contain adequate quantities of calcium.

As it is clear that a vitamin A deficiency without any accompanying calcium deficiency may cause abnormal reproduction, it seems reasonable to attribute the abnormal calvings of the cows on no. 1 timothy hay to the low vitamin A content of this ration rather than to its low-calcium content, even though the vitamin A content was somewhat higher than that of the No. 3 alfalfa ration.

In the case of cow 499, either because her vitamin A requirement was lower than that of the other cows, or because the vitamin A content of her ration happened to be a little higher, reproduction remained normal throughout the period of experimental feeding. The results obtained in her case may, therefore, be taken as a contribution to the study of rations which are rather low in calcium, and, nevertheless, not sufficiently lacking in vitamin A to cause abnormal reproduction.

During the 5 years of timothy hay feeding, she gave about 2,800 kg of milk annually on a ration which contained 25.1 g of calcium daily. She probably lost about 15 percent of the calcium contained in her body at the beginning of the experiment. The milk yield was somewhat lower than that which has been obtained from cows of similar type on No. 1 alfalfa hay, and the writers judge that it was reduced about 10 percent by the timothy hay feeding. The calcium content of the ration was undoubtedly much lower than that of the rations fed on the average dairy farm throughout this country, but by no means as low as may sometimes occur under conditions which prevail in certain districts. The Beltsville grain mixture contained a large proportion of oil meal, and therefore a much higher calcium content than would a mixture made up of cereals and wheat bran, and the timothy hay had a decidedly higher calcium content than is given by Henry and Morrison (*10*) as the average for this roughage. The results definitely suggest that an intake of 25 g of calcium daily is rather low for Jersey cows which are fairly good milkers, and it is not at all

impossible that, if the field is carefully surveyed, as has been done in the study of phosphorus deficiencies (*3*), regions may be found in which the rations contain less calcium than this, and in which calcium deficiency is a serious factor in dairy practice.

Some beneficial results have been obtained by adding calcium salts to rations which were low in their natural calcium content (*1*, *2*, *22*). But, in view of the fact that timothy hay is low in vitamin A as well as in calcium, while alfalfa contains more liberal quantities of both of these essentials, it seems wise at the present time to include a large proportion of legume hay of good quality in the winter dairy ration, wherever possible, and thus to insure at the same time against calcium and vitamin A deficiency. The question whether it may be economical, under certain circumstances, to feed timothy hay, and to provide additional calcium and vitamin A separately in the form of such materials as bone meal and carrots, is a matter for future investigation.

SUMMARY

The balance experiments carried out in the past have been rather contradictory and confusing in regard to certain features of calcium and phosphorus metabolism. Some results suggest that milking cows are likely to be in negative calcium balance, even on rations high in calcium, and that they generally do not utilize more than 20 to 30 percent of the calcium intake for the production of milk and calves. Other results suggest that they may often be in positive balance, even on rations low in calcium, and that they frequently utilize from 40 to 60 percent of the calcium contained in such rations for the production of milk and calves. Certain recent results reported from the Vermont Agricultural Experiment Station indicate that liberally milking cows may store large amounts of calcium and phosphorus in the course of several years on rations low in calcium, and that the proportions of calcium and phosphorus stored may be quite different from those which are contained in normal bone.

In order to obtain light on all these questions, the calcium and phosphorus metabolism of cattle has been studied by slaughtering them and making analyses of their bodies and bones after they had been fed for long periods rations containing different amounts of calcium. The results obtained in these body analyses are compared with the normal calcium and phosphorus content of the fat-free bodies of cattle as shown by analyses carried out in the past. The results of this part of the work may be stated as follows:

(1) The percentages of calcium and phosphorus contained in the fat-free bodies of mature normal cattle are quite constant, and the Ca/P ratio, both in the whole body and in bone, is highly constant.

(2) Keeping cows on low-calcium rations for long periods alters the above relationships less than has often been thought. The Beltsville results indicate that after periods of several years on rations containing only about 25 g of calcium daily, the calcium content of the fat-free bodies of dairy cows is not likely to be reduced to less than 85 percent of the normal level. The change in the Ca/P ratio for the whole body produced by such treatment is even smaller, namely, from 1.9 to about 1.8.

(3) Cows which have been fed low-calcium rations for 9 months or more readily utilize about 50 percent of the calcium intake for the

production of milk and calves. This conclusion is confirmed by the results of balance experiments reported in this paper, in which the calcium and phosphorus balances of cows were determined after they had been fed low-calcium rations for long periods and were still on such rations.

(4) There is every reason to believe that the results of certain balance experiments which suggest that cows may store calcium and phosphorus in proportions entirely different from those usually found in the body, are based on experimental error. The conclusions drawn from other experiments to the effect that cows fed rations high in calcium are usually losing calcium from their bodies; and from still others, that cows fed rations low in calcium may sometimes be storing large quantities of calcium, are also either based on experimental error or due to the fact that the results are not representative of what occurs in long periods.

The work in which calcium and phosphorus metabolism have been studied by means of balance experiments is considered in relation to that in which the general health and performance of milking cows have been studied while they were kept for long periods on rations containing different quantities of calcium. It appears that, when the calcium content of a ration is reduced by substituting timothy hay or straw for alfalfa, the vitamin A content is also likely to be reduced, and that the failures in reproduction, which have occurred on rations in which the roughage was timothy hay or straw, are to be attributed to a vitamin A deficiency rather than to calcium deficiency. The physiological effects of rations which are deficient in calcium, though adequate in vitamin A, are in need of further investigation. Some of the results of the present study may be taken as the beginning of such an investigation. They suggest that, for Jersey cows which are capable of giving 3,000 kg of milk or more annually, an intake of 25 g of calcium daily is somewhat inadequate.

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RESPONSE OF THE WOODY PLANTS HIBISCUS SYRIACUS, MALVAVISCUS CONZATTII, AND BUGINVILLEA GLABRA TO LENGTH OF DAY[1]

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INTRODUCTION

Since 1920 an extensive literature has developed, showing the responses of a great variety of plants to length of day. For the most part the small annual or perennial herbaceous plants have been used, and very little work has appeared to illustrate the flowering response of the woody plants except in the case of the poinsettia (*Euphorbia pulcherrima* Willd.). It is a relatively simple matter to handle the smaller herbaceous plants, whose blooming responses are usually shown during the first or second year from seed. The problem becomes much more difficult when the larger trees and shrubs are considered, for the small container and the usual type of convenient small dark chamber suitable for the small herbaceous plants will not serve for the woody plants.

The shrubs chosen for this study were the turkscap hibiscus (*Malvaviscus conzattii* Greenman), the common garden shrub-althea (*Hibiscus syriacus* L.), and bougainvillea (*Buginvillea glabra* Choisy). The shrub-althea has shown itself well adapted to a study of length of day in all respects. It flowers the first or second season from seed, and is readily grown and handled in small containers. The bougainvillea is also highly responsive to length of day, flowering quickly in summertime in response to artificially shortened daylight exposures.

METHODS

It was at first thought that the flowering responses of shrub-althea could be readily determined by the localization method, which required the construction of small ventilated dark cases over branches of flowering individuals long established in the soil out of doors. In 1929 such a case was constructed over a branch at the Arlington Experiment Farm, Rosslyn, Va., and 10 hours of daylight each day were given this portion of the plant before visible budding occurred. The treated portion of the plant, however, flowered at the same time as the portion receiving the full length of day. Since even in the annuals, flowering, when once initiated, often tends to persist for some time after the original stimulus of a suitable length of day has been removed, it was thought best to grow the shrub-althea under the various lengths of day from the seedling stage.

Seed sown in a flat October 15, 1930, remained in a coldframe out of doors throughout the winter. The seed germinated April 20, 1931, and the seedlings were potted to thumb pots May 28 and to large

[1] Received for publication Feb. 28, 1935; issued August 1935.

buckets June 19, 3 to 4 seedlings being placed in each bucket. The tests began June 19, the plants receiving 10, 12, 12.5, 13, 13.5, 14, and 14.5 hours of daylight and full daylight each day. Large ventilated dark houses were used to reduce the daily light-exposure periods to 14.5 hours or less. On March 1, 1932, the plants were separated and were grown singly in buckets to afford more room for each individual. The buckets for the various tests were numbered and the responses of the plants to the lengths of day originally selected were observed for several consecutive years.

The turkscap hibiscus was grown from cuttings made January 7, 1932. These when rooted were transferred to 3-inch pots February 1 and to 4-inch pots March 8. On April 15 they were transferred to buckets, a single plant being grown in each bucket. The tests with the plants receiving 10, 12, 12.5, and 13 hours of daylight daily began May 9, when the plants were 18 inches tall. The tests with 13.5, 14, and 14.5 hours of daylight each day began May 18, when the daylight period from sunrise to sunset had increased sufficiently. This wooyd hibiscus, being a tender plant, was grown in a warm greenhouse averaging 70° to 75° F. until the tests began out of doors.

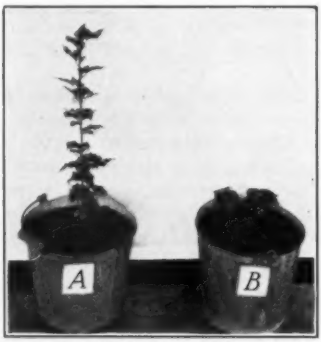

FIGURE 1.—Shrub-althea seedlings 112 days after beginning of tests, which were started May 26, 1930, while plants were in cotyledon stage: *A*, Control receiving full daylight; *B*, plants exposed to 10 hours of daylight each day. Photographed September 15, 1930.

The bougainvillea was grown from young hotbed plants received from Florida in the spring of 1932. These were transferred to large cans, a single plant in each can, and were allowed to grow in the warm greenhouse. The plants flowered heavily during December, attaining the mid-bloom stage in late January during the winter of 1932–33 and again during the winter of 1933–34. On May 16, 1934, long after flowering had ceased and new vegetative shoots had developed, the plants were cut back to 46 inches and placed on the tests. One plant was allowed 10 hours of light daily, another 12 hours of light, and a third was given the full length of day.

EXPERIMENTAL RESULTS

SHRUB-ALTHEA

From the data of table 1 it is obvious that a daily exposure to 10 hours of daylight is too short to allow the shrub-althea to flower, even though the plants have remained for four successive seasons under these conditions. The plants grew very slowly and during the first season remained practically in the rosette condition, forming a very short woody stem only a few inches high upon which thick and abnormally dark green leaves were crowded, as shown in figure 1.

Daily exposure for 12 hours favored greater elongation of the stem, but even this length of day was distinctly unfavorable to flowering. During the seasons of 1931, 1932, 1933, and 1934, only two plants were able to flower, these flowering in 1932, one plant producing a single blossom; the other, three blossoms.

TABLE 1.—*Flowering behavior and height of shrub-althea (Hibiscus syriacus) in four [1] successive seasons under different daily periods of exposure to light from the seedling stage, the daily light exposure for each plant remaining the same throughout the experiment*

Daily light exposure	Plant no.	1932				1933				1934			
		Buds [2]	First bloom	Height	Flowers	Buds [2]	First bloom	Height	Flowers	Buds [2]	First bloom	Height	Flowers
				Inches	*Number*			*Inches*	*Number*			*Inches*	*Number*
10 hours	1			15				21				23	
	2			12				16.5				20	
	3			15				23				28	
	4			24				14				[3]	
12 hours	1			[4]				39		[3]			
	2	June 22	July 18	25	1			33				34	
	3	June 3	June 24	25	3			32				34	
12.5 hours	1	June 20	July 18	26	5	June 26	July 27	31	[3]	June 30	July 25	32	[3]
	2	June 10	July 8	21	10	...do....	July 28	23	[3]	...do....	July 20	39	[3]
13 hours	1	June 14	July 11	37	30	July 10	Aug. 3	45	[3]	July 3	July 31	47	[3]
	2	...do....	[6]	[6]	1	July 17	Aug. 4	40	[3]			[3]	
	3	...do....	July 14	40	14	July 14	July 31	44	[3]	June 30	July 26	48	[3]
13.5 hours	1	June 13	July 11	31	21	June 28	July 28	39	[3]	...do....	July 31	41	[3]
	2	...do....	July 8	41	16	July 6	Aug. 7	45	[3]	...do....	Aug. 4	48	[3]
	3	July 8	Aug. 8	28	17	June 28	July 31	38	[3]	...do....	Aug. 2	43	[3]
	4	July 14	July 6	42	37	June 30	Aug. 4	46	[3]	...do....	July 27	50	[3]
14 hours	1	June 10	July 7	36	34	July 12	Aug. 12	43	[3]	July 7	Aug. 10	40	[3]
	2	June 1	June 25	35	60	June 30	Aug. 4	39	[3]	July 9	...do....	48	[3]
14.5 hours	1	June 20	July 25	45	43	July 12	Aug. 15	44	[3]	July 13	Aug. 29	51	[3]
	2	June 9	July 7	35	35	June 30	July 31	40	[3]	July 8	Aug. 6	53	[3]
	3	June 15	July 18	30	32	...do....	Aug. 4	37	[3]	...do....	Aug. 10	49	[3]
	4	June 1	July 5	29	23	...do....	July 31	40	[3]	July 13	Aug. 27	55	[3]
Full daylight	1	June 21	July 7	23	6	[7]		[4]	[3]	July 11	Aug. 6	29	[3]
	2	June 5	July 5	48	64	July 6	Aug. 10	49	[3]	July 7	July 31	51	[3]
	3	June 21	July 25	43	38	July 3	Aug. 3	50	[3]	July 5	Aug. 4	53	[3]
	4	[6]	June 6	25	42	[6]	[6]	[4]	[3]	[3]			
	5	July 21	Aug. 17	33	10	[6]	[6]	[4]	[3]			[4]	[3]
	6	June 21	July 22	44	27	[6]	[6]	[4]	[3]	Aug. 15	Sept. 15	47	[3]

[1] The data for 1931 are as follows: Plant 1, under 13 hours of daylight, budded Sept. 5, showed first bloom Oct. 5, was 26 inches in height, and had 1 flower; plants 1 to 4, under 10 hours of daylight, attained a height of 3 to 4 inches, but did not flower and were not measured; none of the other plants flowered and none was measured.
[2] Leaders indicate that the plants did not flower.
[3] Plant died.
[4] Not measured.
[5] Many flowers.
[6] Not recorded.
[7] Did not flower; plant unhealthy.

Daily exposure for 12.5 hours allowed flowers to develop, but the plants grew relatively slowly and did not attain a floriferous condition until 1933. A rather weak flowering impulse was evident in 1932. Daily periods of exposure of 13, 13.5, 14, and 14.5 hours were quite favorable to growth and flowering, and about as many flowers were produced as appeared under conditions of full daylight. The mean date of appearance of the first flower for the three seasons 1932, 1933, and 1934 was not materially changed under the different periods of exposure favorable to flowering. For the daily 12-hour exposure the mean date of flowering (which occurred only in 1932) was July 6; for the 12.5-hour day, July 21; for the 13-hour day, July 26; for the 13.5-hour day, July 27; for the 14-hour day, July 27; for the 14.5-hour day, August 2; and for full daylight, July 28.

Growth in height showed a consistent increase with increase in the length of the daily period of illumination. The mean height at the end of 1934 for the 10-hour period of exposure was 23.6 inches; for the 12-hour, 34 inches; for the 12.5-hour, 35.5 inches; for the 13-hour,

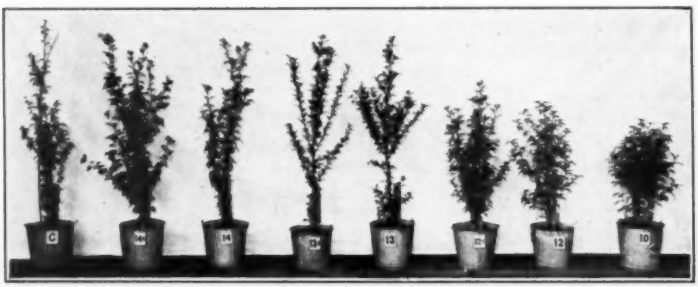

FIGURE 2.—Shrub-althea seedlings grown four seasons under the various daily periods of light exposure indicated. Plant designated *C* received full length of day. Dates of flowering in 1934 under the various periods of exposure: Full day, August 4; 14.5 hours, August 10; 14 hours, August 10; 13.5 hours, August 2; 13 hours, July 26; and 12.5 hours, July 20; 12 and 10 hours, no flowering. Photographed August 7, 1934.

47.5 inches; for the 13.5-hour, 45.5 inches; for the 14-hour, 44.0 inches; for the 14.5-hour, 52.0 inches; and for the conditions of full daylight, 45.0 inches. Figure 2 shows the habit of growth of shrub-althea, under the various daily periods of exposure, at the end of four seasons.

TURKSCAP HIBISCUS

From the data of table 2 it is obvious that the turkscap hibiscus is indeterminate in its responses to length of day, at least for the annual range in day length at Washington, D. C., since it flowered readily under all periods of daylight exposure, from 10 hours to the local full length of day, which on June 21 is about 15 hours from sunrise to sunset. This behavior is illustrated in figure 3. There was some delay in the flowering of some of the plants as the daily periods of exposure to daylight were increased beyond 10 hours. In the initiation of buds the greatest delay occurred under full length of day. Elongation of the stems tended to increase with increase in the length of the daily exposure, the full length of day producing the tallest plants. The plants were more compact and branching in habit of growth under the daily exposure periods of 10, 12, 12.5, and 13 hours. These plants also flowered more freely.

FIGURE 3.—Turkscap hibiscus. A woody subtropical plant flowering freely under all lengths of day, from 10 hours or less to the full length of day, at Washington, D. C. The plants were grown from cuttings made January 7, 1932. Tests for the daily exposure periods of 10, 12, 12.5, and 13 hours began May 9; for periods of 13.5, 14, and 14.5 hours, May 18. The control (*C*) received the full length of day. The number of days required for budding and first flowering are shown in table 2. Photographed July 18, 1932.

TABLE 2.—*Number of days required for production of visible buds and first flowering by turkscap hibiscus (Malvaviscus conzattii) and the height attained by the plants when exposed to various day lengths, beginning in May 1932*

Daily light exposure	Days required for production of—		Height of plants	Daily light exposure	Days required for production of—		Height of plants
	Buds	First flowers			Buds	First flowers	
	Number	*Number*	*Inches*		*Number*	*Number*	*Inches*
10 hours	29	54	31	13.5 hours	23	54	42
12 hours	32	63	37	14 hours	28	54	47
12.5 hours	32	63	42	14.5 hours	28	57	44
13 hours	32	63	44	Full day	50	63	55

BOUGAINVILLEA

Whereas the shrub-althea behaved as a typical long-day plant, the bougainvillea flowered best in the short daily light periods. Growth of new shoots in the latter began at once in all the tests. The plant receiving 10 hours of daylight each day showed buds June 28, and flowered July 9 at a height of 75 inches. The plant was very floriferous, and quite as gorgeous with brilliant pink flowers as it has been in February when it flowered in response to the natural short days of the winter time. The plant receiving 12 hours of daylight each day budded July 25 and flowered August 4 at a height of 82 inches and was much less floriferous than the plant receiving 10 hours of daylight each day. The controls showed no indications of budding in early October. This behavior of flowering in summertime in response to shortened daily periods of exposure is shown in figure 4. Bougainvillea normally flowers at Washington, D. C., in the greenhouse in December when the length of day from sunrise to sunset is less than 10 hours.

DISCUSSION

Shrub-althea, a woody plant, behaves as a typical long-day plant when grown from the seedling stage and subjected to different daily periods of exposure to daylight in summertime, since flowering is favored by an increasing length of day. A daily exposure of 10

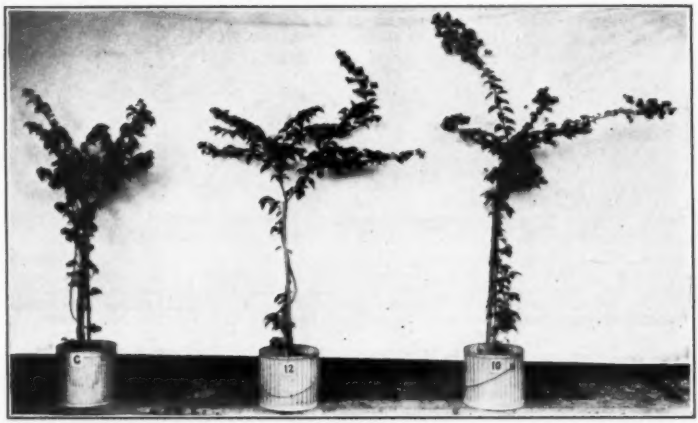

FIGURE 4.—*Bugainvillea glabra* grown with 10 and 12 hours of daylight each day and with full length of day (*C*). The tests began May 16, 1934, at which time the plants were cut back to 46 inches. The plant receiving 10 hours of daylight each day budded June 28, and flowered July 9 at a height of 75 inches; the plant receiving 12 hours of daylight each day budded July 25, and flowered August 4 at a height of 82 inches; the plants receiving the full daylight had not budded in late October. Flowering normally takes place in December, as shown in figure 5. Photographed August 7, 1934.

FIGURE 5.—Bougainvillea flowering in the greenhouse in response to the short days of the wintertime. The first flowers appeared in December 1933 and the plant was in full bloom February 1, 1934. This is one of the plants shown in figure 4 and was induced to flower a second time by reducing the length of day. Photographed February 16, 1934.

hours entirely inhibits flowering and greatly retards growth. The plants grew slowly, being compact and much branched, with a very stocky main stem and short thick branches. A daily exposure of 12 hours is distinctly unfavorable to flowering, but growth is improved.

The turkscap hibiscus is decidedly indeterminate in its flowering response, since it flowers under all lengths of day from 10 hours of daylight to the full length of day at Washington, D. C. For this reason it finds a place as a useful garden ornamental in Florida and other warmer parts of the United States. It flowers readily far northward in summertime, and continues to be floriferous under greenhouse conditions in wintertime. However, being a tender shrub, it is not suitable for outdoor culture where severe winter weather prevails.

Bougainvillea, flowering best on a shortened length of day, behaves as a typical short-day plant. This is indicated by its natural tendency to flower only in midwinter, when the days are shortest, and from the fact that it remains in a flowerless vegetative condition in summertime. In this latitude a day length of only 10 hours is not attained until November 16. The winter flowering behavior is shown in figure 5.

These tests with shrub-althea and the tropical bougainvillea indicate that both woody plants are quite as sensitive to different lengths of day as those annual and perennial herbaceous plants which have been classed as typical long-day or short-day plants.

The poinsettia, also a woody plant, early received attention.[2] It is obvious that the bougainvillea and the poinsettia have a very similar type of behavior, both flowering when the days are very short. They may be regarded as typical short-day woody plants, since both flower on a shortened length of day. Both are native to the tropical and subtropical regions of the Americas, the bougainvillea being a native of Brazil, the poinsettia of the warmer parts of Mexico and Central America. From the fact that their flowering responses are rigidly dependent upon daily exposures to daylight for 12 hours or less, these plants normally have no horticultural value as flowering plants in northern latitudes except under warm greenhouse conditions in wintertime.

The shrub-althea, a long-day plant, attains flowering on a lengthened day, and because it is floriferous when the summer days are very long becomes useful as a garden ornamental in far-northern latitudes in summertime. A plant which is useful both in the Tropics and under outdoor conditions in high latitudes must be of the indeterminate type with respect to length of day.

SUMMARY

Shrub-althea (*Hibiscus syriacus* L.) behaves in response to length of day as a typical long-day plant, i. e., it flowers in response to a lengthened day. Exposure to 10 hours of daylight each day does not allow flowering. Daily exposure for 12 hours is unfavorable to flowering. Flowering takes place under all lengths of day from 12 or 12.5 hours to and including at least the full length of day of 15 hours in the Washington, D. C., region, but abundant flowering is associated

[2] GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

with the longer periods of exposure. Growth and elongation are also favored by the longer daily exposure.

The turkscap hibiscus (*Malvaviscus conzattii* Greenman) is indifferent or indeterminate in its response to different lengths of day within the range covered by the tests. The plants are more compact in habit of growth and more floriferous under the shorter daily exposure periods, i. e., 10, 12, 12.5, and 13 hours.

Bougainvillea (*Buginvillea glabra* Choisy) behaves as a typical short-day woody plant, flowering on shortened lengths of day. Even in midsummer, when the plants are strictly vegetative, flowering is quickly initiated by daily exposure to 10 hours of daylight. Under the same conditions exposure to 12 hours of daylight each day was distinctly less favorable to flowering.

The day-length requirements of the woody plants shrub-althea, bougainvillea, turkscap hibiscus, and poinsettia are now fairly well understood. The shrub-althea, bougainvillea, and poinsettia are quite as sensitive to the factor of length of day as any of the herbaceous plants. It is evident that the usefulness of all these plants for certain seasons and regions depends as much upon their particular length-of-day requirements as upon any other factor.

PLEOSPORA ROT OF TOMATOES[1]

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INTRODUCTION

In the investigation of the various diseases causing losses of fruits and vegetables during transit, storage, and marketing, a new or unusual fungus is sometimes encountered on produce from a more or less localized district. Whether the causal organism is just becoming established in that locality, whether certain new host relationships account for the initiation of the disease, or whether the immediate climatic conditions happened to favor the growth of the organism is often difficult to determine. Pleospora rot of tomato (*Lycopersicon esculentum* Mill.) furnishes an interesting example of a disease induced by an organism known to have been present in the Salinas district of California for at least 11 years without causing serious damage, but later, for reasons not understood, becoming an important factor in the shipping of tomatoes from that district.

In November 1919, a few tomatoes showing an unusual type of decay were obtained from cars of California tomatoes received on the Chicago market. At that time the disease was of little economic importance but was of scientific interest since a *Pleospora* with an associated *Macrosporium* was isolated from the advancing edge of numerous lesions, and a search through the literature failed to reveal any mention of a pleospora rot of tomatoes. Cultures of this fungus have been kept in stock since that time, and each year during November and December other cultures of the organism have been isolated from tomatoes showing similar symptoms on arrival at the Chicago market.

For the past 3 years this disease has been of great economic importance to the California tomato crop grown for marketing during the early winter months. So far as has been observed the disease is not important in those tomato-growing districts of California that ship in other seasons of the year, and it has not been found elsewhere except in Mexican stock shipped during January. Within the past 2 years a few specimens of pleospora rot have been obtained from shipments of Mexican tomatoes arriving on the Chicago and New York markets.

Two hundred and forty-eight isolations of species and strains of *Alternaria* and *Macrosporium* have been made from tomatoes received from practically all of the tomato-producing sections of the country; but, with the exception of the *Macrosporium* having the *Pleospora* stage herein reported, none of them have developed the perfect stage either on the fruit or in pure culture. There are numerous reports of *Alternaria* and *Macrosporium* species associated

[1] Received for publication Apr. 13, 1935; issued August 1935.

with various types of decay in tomatoes. As indicated by Ramsey and Link (*7*),[2] many of these organisms are found following other diseases such as phoma rot, blossom-end rot, and sun scald, while others appear primarily as wound parasites following growth cracks or mechanical injuries, or in the stem scar. These fungi are especially destructive to tomatoes during long transit periods, on track, or in the ripening rooms on the receiving markets.

McWhorter (*4*) in Virginia and Samuel (*8*) in South Australia have described a stem-end rot of tomatoes induced by *Macrosporium solani* Ell. and Mart., and Douglas (*2*) in California reported a new alternaria spot of tomatoes caused by an organism which he believed differed from both *M. solani* and *M. tomato* Cke. The types of lesions described in these reports are similar in some respects to those produced by the *Macrosporium* stage of the *Pleospora* isolated from California tomatoes. However, no perfect stage was observed by the investigators mentioned, and a study of the conidia readily shows the difference between these fungi and the one described in the present paper.

ECONOMIC IMPORTANCE

The best idea as to the seriousness of pleospora rot of tomatoes may be obtained from a study of the inspection reports made by the Food Products Inspection Service of the Bureau of Agricultural Economics, United States Department of Agriculture, on the condition of tomatoes arriving on the larger markets. From these reports it is apparent that the amount of decay induced by *Pleospora* varies greatly from season to season and also with the length of the haul. Decay develops slowly in green tomatoes during the first 3 or 4 days in transit and then makes more and more rapid headway as the fruits begin to ripen. Consequently, the tomatoes arriving on the New York market and other eastern markets often show from 10 to 25 percent more pleospora rot than similar stock received on the Chicago market.

Upon arrival in New York in November 1931, a car of California tomatoes showed 90 percent of the fruit infected at the stem end with *Pleospora*. Another car of tomatoes arrived on this market showing only 2 percent decay, but after the tomatoes had been allowed to ripen in the car on the tracks for almost 2 weeks 50 to 60 percent of them showed serious decay induced by *Pleospora*. A car of California tomatoes that passed shipping-point inspection, containing 85 percent U. S. No. 1 tomatoes with no appreciable decay, was found to have 25 to 80 percent, or an average of 55 percent, pleospora rot when the stock was finally marketed in New York. Numerous cars of California tomatoes shipped during November and December in the last 3 years have shown from 25 to 50 percent loss on account of this disease. The mature, good-quality stock ripens during transit or soon enough after arrival to escape great loss, but the immature or poor-quality stock, which ripens slowly and must often be held for a week or more for ripening, is subject to very heavy decay.

[2] Reference is made by number (italic) to Literature Cited, p. 42.

THE DISEASE

DEVELOPMENT AND SPREAD

No study has been made of the development and spread of pleospora rot in the field; consequently, no data are at hand concerning its prevalence on growing tomato plants. From studies of the early stages of the disease as found in commercial shipments received on the Chicago market and from correspondence with Federal-State shipping-point inspectors, it is evident that close observation is necessary to detect the presence of early infections about the stem scar of green tomatoes. It appears probable that the fruit pedicels and calyx sometimes harbor the fungus and that under favorable moisture and temperature conditions the organism works its way from this source down into the fruit. In wet weather or during fogs spores lodging under the calyx may also germinate and penetrate the tissues at the edge of the stem scar before the fruit is harvested, or follow immediately in wounds incident to harvesting. There is no evidence that the fungus spreads through the wrappers from one tomato to another during transit.

SYMPTOMS

Small brown V-shaped to oval spots at the edge of the stem scar or in mechanical wounds elsewhere on the fruit are the first visible symptoms of pleospora rot (pl. 1, *A*, *B*). These lesions appear somewhat similar to those produced by *Phoma*, but as they enlarge the brown color is retained and usually a small amount of gray to grayish-brown mycelium is noticeable over the lesion, whereas in typical *Phoma* lesions of the same size the affected tissues are black and there is no visible surface mold. Spots one-half inch or more in diameter generally show the black pimplelike perithecia forming in the central region. Tomatoes arriving on the market after a 5- to 8-day transit period show lesions varying from one-fourth to three-fourths of an inch in diameter. As the tomatoes ripen the fungus progresses rapidly, and a moderately firm greenish-brown to brown decay may involve one-fourth of a fruit within 10 days after it is shipped (pl. 1, *B*, *C*). The greatest losses are sustained in shipments of tomatoes that must be held on track or in the humid ripening rooms for several days before they ripen enough to be placed on the market. Under these humid conditions there is usually an abundant development of mycelium which characterizes the *Macrosporium* stage of this fungus (pl. 1, *C*, *E*). The black perithecia of *Pleospora lycopersici* are prominent in the older lesions (pl. 1, *E*).

Typical *Pleospora* lesions show evidence that the fungus entered through or at the edge of the stem scar. The organism penetrates into the fruit one-half inch or more, causing a moderately firm dark-brown decay such as that shown in plate 1, *D*, and in many instances it discolors the vascular elements underneath the scar and along the side walls of the fruit. When infection takes place through wounds on the sides of the tomato the vascular elements are not discolored.

INOCULATION EXPERIMENTS

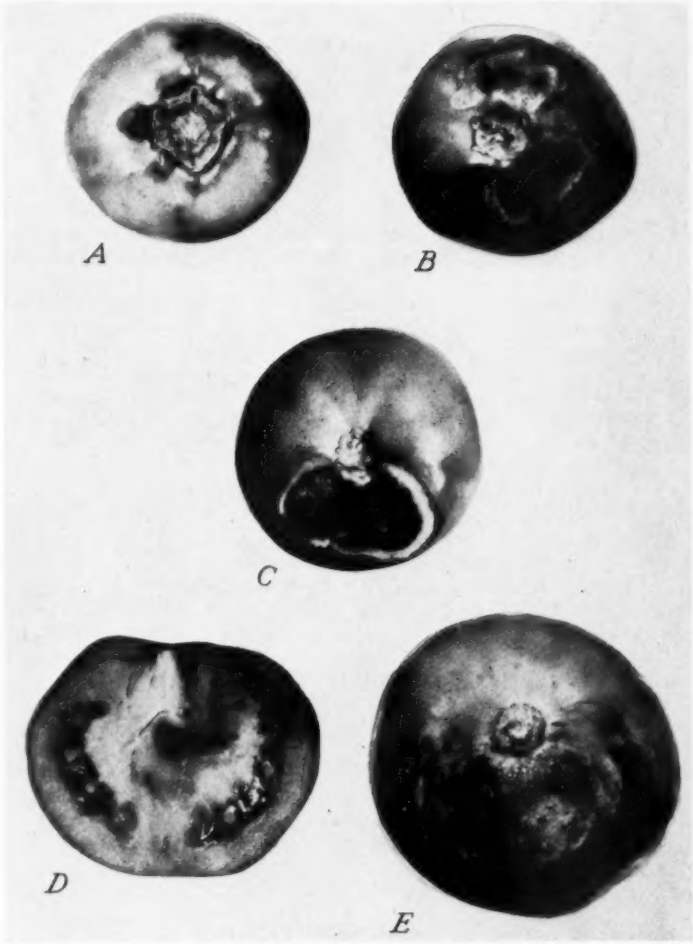
The pathogenicity of both the *Pleospora* and *Macrosporium* stages of this fungus is readily established by inoculation experiments. Mature-green tomatoes, selected for freedom from disease and blem-

ishes, were sterilized in a solution of formaldehyde (1 to 240) for 3 minutes and then rinsed in sterile water before inoculations were made. In each experiment several tomatoes were inoculated by placing mycelium and spores in small punctures through the skin of each fruit. The results obtained from such experiments show that the decay induced progresses slowly in green tomatoes and that as the fruits ripen the development of the lesions becomes more and more rapid. Changes in the chemical composition of the fruit during ripening influence the rate of growth of the fungus. Changes in acidity of the fruit juices probably play an especially important part. Commercially mature-green tomatoes are somewhat acid (pH 4.7), while tomatoes ripe enough for table use are much less acid (pH 6.01).

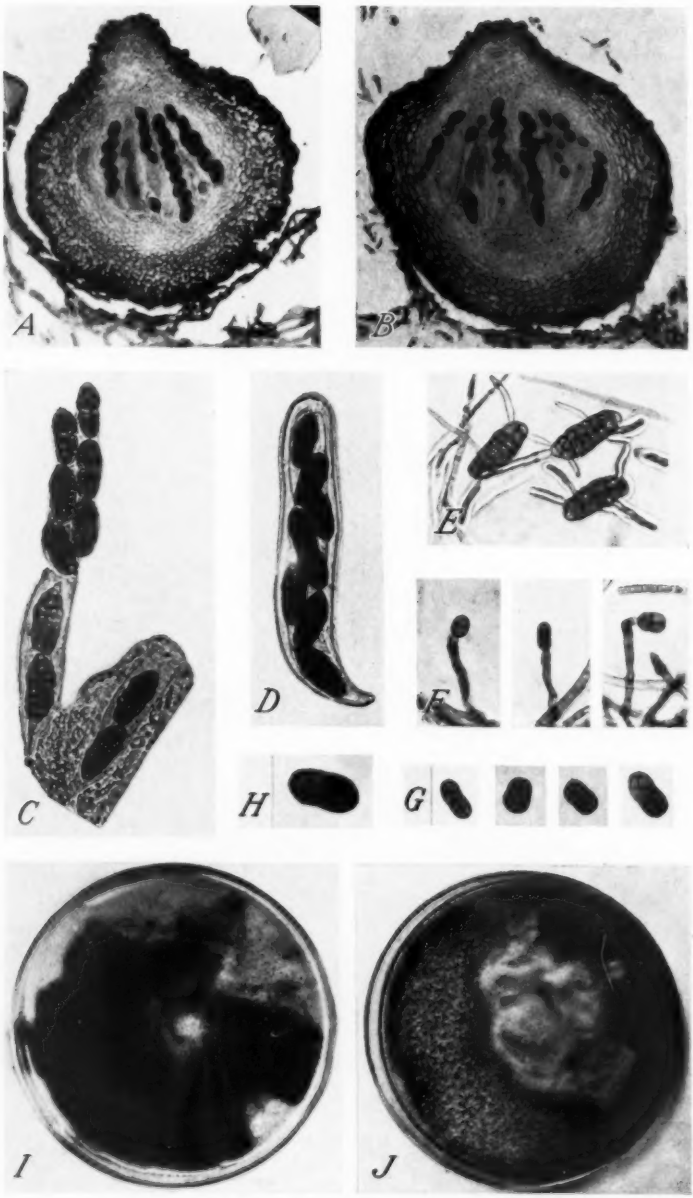
Mature-green tomatoes inoculated as described above and held at 45° F. develop lesions that can barely be detected within 10 days, and at 55° the lesions reach an average diameter not much greater than one-fourth of an inch in 10 days. The fungus becomes more active at 65°, and lesions one-fourth of an inch in diameter are produced in a week and lesions five-sixteenths of an inch in diameter within 10 days. On cutting the fruit held under these conditions it was found that the average diameter of the lesions within the locules was one-half inch. At 70°, the temperature at which most tomatoes are held for ripening, the average internal diameter of lesions developed in 10 days is about three-eighths of an inch, whereas the external lesions are about one-fourth of an inch. The rate of enlargement of lesions begins to slow down at 75°, few spots reaching a diameter greater than one-fourth of an inch and a depth of five-sixteenths of an inch within 10 days. In mature-green tomatoes held at 85° no development of decay could be detected in 10 days.

Ripe tomatoes inoculated with *Pleospora* and held at 65° F. develop lesions almost one-half an inch in diameter in 1 week and a little less than five-eighths of an inch in diameter in 10 days. At 70° decayed areas one-half inch in diameter are developed in a week, and these lesions are approximately five-eighths of an inch in diameter within 10 days. Above 70° the rate of enlargement of lesions diminishes rather rapidly. At 75° lesions develop only one-fourth of an inch in diameter in a week, and within 10 days most spots do not reach a diameter greater than five-sixteenths of an inch. Only slight development of decay can be detected after 10 days in fruits held at 80°.

Because of variations in the manner of growth of *Pleospora* in inoculated tomatoes and unavoidable variations in the maturity of the fruits, the size of the lesions developed does not always indicate accurately the effects of the different temperatures upon the fungus. In some instances the fungus grew extensively inside the seed cavity while the external lesions remained relatively small. In making final readings on inoculated tomatoes the lesions were cut and cognizance was taken of the development in the interior of the fruit. For the direct effect of temperature changes upon the growth rate of the fungus reference should be made to figure 1, which shows the diameters of the fungus colonies that developed on the flat surface of potato-dextrose agar in Petri dishes held at the specified temperatures. The growth on artificial media does not correspond exactly with that made in the tomato fruit, but it is interesting to note that the minimum, optimum, and maximum temperatures for the growth of the organism



A, Mature-green tomato showing the early stages of pleospora rot about the stem scar; *B*, mature-green tomato showing two V-shaped early stages of decay at the edge of the stem scar, and an advanced stage with gray surface mycelium; *C*, mature-green tomato showing the greenish-brown decay and well-developed grayish-brown mycelium of the *Macrosporium* stage characteristic of tomatoes held on track or in the moist atmosphere of ripening rooms; *D*, section through stem end of mature-green tomato showing well-developed greenish-brown to brown decay and slight discoloration of the vascular elements underneath the stem; *E*, advanced stage of decay of ripe tomato showing mature black perithecia.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

agree rather closely with the results obtained in the inoculation experiments.

THE CAUSAL ORGANISM

IDENTITY

A check of the measurements of the *Pleospora* isolated from California tomatoes indicates that it is identical with *Pleospora lycopersici* É. and Ém. Marchal (*5*), which was described in 1921 as a new fungus causing a fruit rot of tomatoes in Belgium. The conidial stage, determined as *Macrosporium sarcinaeforme* Cav.,[3] was also observed at that time, but no description was given of the type of lesions produced.

The measurements for the California strain of *Pleospora lycopersici* as developed on potato-dextrose agar are as follows: Perithecia, 265μ–375μ×325μ–550μ average 325μ×425μ; asci, 22.5μ–32.5μ× 125.5μ–192.5μ, average 28.2μ×167.0μ; ascospores, 12.5μ–19.0× 30.0μ–41.0μ, average 15.2μ×34.4μ; conidia, 11.0μ–15.0μ×20.0μ–36.5μ, average 13.5μ×26.0μ.

Except for a previous report by the writer (*6*), apparently no mention has been made of the occurrence of this fungus in the United States.

DEVELOPMENT

When the perithecia become moist or are placed in a drop of water the asci exude through the ostiole. As they emerge they absorb water very rapidly and by breaking through a thin place in the apex of the outer wall (pl. 2, *D*) the inner flexible wall elongates until the ascus is about twice its original length (pl. 2, *C*). The thicker, somewhat gelatinous inner wall continues to absorb water until the internal pressure becomes so great that it breaks. This wall usually ruptures, either at the tip of the ascus or just above the ring (pl. 2, *C*) made by the tip of the outer wall after it contracts towards the base of the ascus, as described by Atanasoff (*1*) for *Pleospora herbarum* (Pers.) Rabh. In the second instance the ascospores are expelled laterally, while in the first the spores are ejected singly through the small orifice at the apex much after the manner of shooting Roman candles. Hodgetts (*3*) describes a similar method of spore discharge in *Leptosphaeria acuta* (Moug.) Karst. The ascospores and conidia of *P. lycopersici* germinate readily in water and in tomato juice. In water at room temperature the germ tubes of ascospores develop within 3 hours to the extent shown in plate 2, *E*.

The drawings by Él. and Ém. Marchal (*5*) show the conidia to be smooth-walled. The epispores of the conidia belonging to the *Pleospora* isolated from California tomatoes are minutely echinulate. In some stages of development the conidia appear smooth, but characteristically the epispore is definitely echinulate (pl. 2, *F*, *G*, *H*). This character, together with the sarcinaeform type of spore, would place the conidial stage in the genus *Thyrospora* as established by Tehon and Daniels (*9*), in which *Thyrospora sarcinaeforme* (Cav.) comb. nov. is made the type species.

EXPLANATORY LEGEND FOR PLATE 2

A, Longitudinal section through a mature perithecium of *Pleospora lycopersici*. *B*, Longitudinal section through a perithecium showing some stages in the development of asci and spores. *C*, Ruptured ascus showing six spores in the gelatinous matrix of the inner wall and two spores remaining in the foot of the ascus. The ring made by the broken outer wall, which contracts toward the base of the ascus, is shown just below the group of six spores. *D*, Mature ascus before absorption of water and elongation by rupture of outer wall. The small white V-shaped notch in the tip of the ascus marks the thin place in the wall at which rupture occurs. *E*, Ascospores germinated in water. *F*, Conidiophores and conidia showing three developmental stages. *G*, Four mature conidia. *H*, Mature conidium greatly enlarged to show echinulation of the spore wall. *I*, Plate culture of *P. lycopersici* showing white saltant sectors, which produce perithecia but not conidia. *J*, Plate culture of *P. lycopersici* showing the rapidly developed gray mycelium of the *Macrosporium* stage in contrast with the white to light-gray slower growing mycelium of the *Pleospora* stage.

CULTURE STUDIES

Development of Colonies

Cultures grown from single ascospores on potato-dextrose agar usually produce conidia (pl. 2, *F*, *G*) within a week, and shortly thereafter numerous perithecia begin to form (pl. 2, *I*). Mature ascospores are not found within the perithecia (pl. 2, *A*, *B*) until the cultures are 2 to 3 weeks old. Colonies grown from single conidia develop in the manner described above, both conidia and ascospores being formed in the usual length of time required for plantings made from mycelium and ascospores.

Cultural Characters

This fungus, when grown on potato-dextrose agar slants in test tubes, can be separated readily from ordinary *Alternaria* and *Macrosporium* cultures by the rose-colored crescent formed at the top of

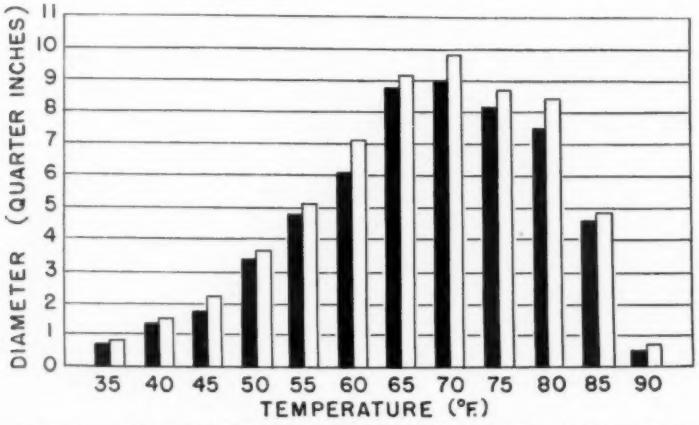


Figure 1.—Average diameters of colonies of *Pleospora* developed in 7 days on potato-dextrose agar pH 4.7 (black bars) and pH 6.01 (light bars) at specified temperatures.

the slant. This colored crescent is 2 to 4 mm wide, and the pigment does not diffuse into the agar as is characteristic of the chromogenic Alternarias. Occasionally certain strains grown in plate cultures at high temperature develop a slight yellowish diffusible pigment, but this is not common for the organism, whereas the rose crescent is characteristic. So far, it has been the writer's experience that all *Macrosporium* cultures that when isolated from tomatoes and other hosts developed this rose crescent have produced a *Pleospora* stage.

Cultures of *Pleospora lycopersici* developed from single spores have a marked tendency to produce saltant sectors such as that shown in plate 2, *I*. Some saltants, like the white V-shaped areas, develop rudimentary perithecia which seldom produce well-formed asci and spores. The remaining dark-gray, more rapidly growing part of the colony bears conidia and few perithecia. In plate 2, *J*, is shown a white cottony sterile growth in the center of the colony and a well-developed gray *Macrosporium* stage bearing conidia in great abundance.

Effect of Acidity and Temperature on Growth Rate

In figure 1, which shows graphically the diameters of colonies of *Pleospora* that developed in 7 days at different temperatures, data are given on the effects of acidity upon the growth rate of the fungus on an artificial medium. In all experiments of this kind a potato-dextrose agar was used. For direct comparison studies a large amount of this medium was made and then divided into two lots; one lot was titrated to pH 6.01 and the other to pH 4.7. These acidities were arrived at by testing numerous mature-green tomatoes and tomatoes that were ripe enough to be in prime condition for salads. The average pH value of the mature-green fruits was 4.7, whereas that of the ripe tomatoes was 6.01.

The height of the black columns in figure 1 represents the weekly growth in diameter of the fungus culture on potato-dextrose agar of the acidity of mature-green tomatoes (pH 4.7); the light columns indicate the corresponding growth made on agar of the acidity of ripe tomatoes (pH 6.01). In this figure it is readily seen that at all temperatures at which appreciable development was made the fungus grew more rapidly on the less acid medium. This is in complete accord with transit and market observations made on naturally infected and on artificially infected tomatoes, all of which showed more rapid decay in the ripening fruits. It should be noted, however, that the difference in the amount of decay made on green and ripe fruits is proportionately much greater than the difference between the size of the colonies developed on agar at pH 4.7 and 6.01 at corresponding temperatures. A probable explanation of this difference in the rate of development of the organism is that changes other than those in acidity, made during the ripening of the fruit, are also important in influencing the growth rate and consequently the amount of decay induced by this organism.

The minimum temperature for the development of *Pleospora lycopersici* on potato-dextrose agar (pH 4.7 and 6.01) is approximately 35° F., and the maximum is 90°. Slight growth is made at these temperatures for the first 2 or 3 days and then all development ceases, although the organism is not killed at either of these extremes. The optimum temperature for the development of this fungus is 70°. As indicated in the graph, very poor growth is made at all temperatures up to 55° and above 85°, while at the temperatures at which tomatoes are shipped and ripened (60° to 80°) growth is favored. Change in the growth rate could be detected whenever the fungus showed a marked tendency toward developing either the conidial stage or the perfect stage to the exclusion or near exclusion of the other. The conidial stage, *Macrosporium sarcinaeforme*, always grew faster at higher temperatures, and its optimum was about 5° higher (75°) than that for the *Pleospora* stage. The colony illustrated in plate 2, *J*, which was developed from a single central planting, shows the variability in growth rate of the different stages of this organism.

SUMMARY AND CONCLUSIONS

Pleospora rot of tomatoes was first recognized in the United States in a car of California tomatoes received on the Chicago market in November 1919. Pure cultures of the causal organism were obtained at that time, and since then numerous other isolations of the fungus

have been obtained. Pleospora rot has been found only in California tomatoes marketed during November and December and in Mexican stock shipped in January.

The disease induced by *Pleospora* has become increasingly important during the past 3 years. Losses of as high as 50 to 90 percent of the tomatoes in some cars have been reported. Infections may take place in the tomatoes while still in the field or during harvesting and packing. Decay develops slowly during the first 3 or 4 days in transit while the fruits are green, but as they ripen decay progresses more rapidly. Spots varying from one-fourth to three-fourths of an inch in diameter may develop during transit. Greenish-brown to brown moderately firm decayed areas involving one-fourth of a fruit have been developed in ripening tomatoes within 10 days after shipment. The *Macrosporium* stage is evident on the fruits at the time of arrival on the markets, and in most lesions one-half an inch in diameter the *Pleospora* stage is also evident.

Mature-green tomatoes inoculated with *Pleospora lycopersici* and held at various temperatures show little or no decay below 45° F., or above 80°. The greatest development of decay takes place at 65° to 70°. Decay developed more rapidly in inoculated ripe tomatoes at all temperatures, but the minimum, optimum, and maximum temperatures were about the same as for green fruits.

The fungus isolated from California tomatoes has been found to be identical with *Pleospora lycopersici*, which was described in 1921 by Él. and Ém. Marchal as a new fungus causing decay of tomatoes in Belgium. The conidial stage, *Macrosporium sarcinaeforme* Cav., was reported by them, and it has been found constantly associated with the *Pleospora* stage in California tomatoes. Single-spore cultures made from either ascospores or conidia give rise to both the *Pleospora* and *Macrosporium* stages of the fungus.

On potato-dextrose agar (pH 4.7 and 6.01) the minimum temperature for growth of the fungus was 35° F., the optimum 70°, and the maximum 90°. In cultures having a distinct tendency toward producing the *Macrosporium* stage the growth rate was more rapid than in those cultures in which the *Pleospora* phase was dominant. The optimum temperature for the development of the *Macrosporium* stage alone was about 75°. At all temperatures at which appreciable development of the fungus was made, the growth rate was more rapid on the agar having a pH value of 6.01 (the average acidity of ripe tomatoes) than on one having a pH value of 4.7 (the average acidity of mature-green tomatoes). This harmonizes with the fact that most rapid and serious decay induced by *Pleospora lycopersici* takes place on the market in tomatoes that are in the turning and ripe stages.

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A CHEMICAL INVESTIGATION OF THE FERMENTATIONS OCCURRING IN THE PROCESS OF POI MANUFACTURE[1]

By LEONORA NEUFFER BILGER, *collaborator in chemical research*, and HONG YIP YOUNG, *assistant in chemical research, Hawaii Agricultural Experiment Station*

INTRODUCTION

In a bulletin of the Hawaii Agricultural Experiment Station (*2*) [2] Allen and Allen discussed, from the bacteriological standpoint, the fermentations occurring in the preparation of poi. They concluded that the fermentation of poi might be divided into two phases, the first, covering 5 or 6 days, in which bacteria of a high acid-producing type predominated; and the second, extending from the third to the sixth day, in which a flora of yeasts, mycoderms, and oidia was increasingly prevalent. This paper reports the results of an attempt to identify chemically the products of bacterial action shown by Allen and Allen to occur in poi at different stages of fermentation.

So far as the specific products of fermentation are concerned, the study resulted in qualitative determinations only. Attempts to secure quantitative data led to the conclusion that the physical nature of poi, variations in samples, exceedingly small quantities of fermentation products, and the effect of even very slight variations in conditions upon such small quantities rendered quantitative determinations practically impossible. The quantities could only be roughly indicated.

Preliminary to the identification of the specific products of fermentation, quantitative determinations were made of starch, reducing sugars, volatile acids, and nonvolatile acids in fresh poi [3] and in poi at various stages of fermentation. These data proved of value in predicting the products of the chemical and bacteriological changes. Quantities of fresh poi were obtained as needed from a poi factory in Honolulu. Attempts to use frozen poi were abandoned early in the work because it was found difficult to secure the uniformity of samples necessary for analytical purposes.

ANALYSIS OF FRESH POI

Methods used in the analysis of fresh poi for moisture, starch, pentosans, reducing sugars, sucrose, proteins, fat, crude fiber, ash, phosphorus, and calcium were standard methods taken, in the main, from Allen (*1, v. 1*), Official and Tentative Methods of Analysis (*3*), and Browne (*4*). Reducing sugars were determined by McAllep's

[1] Received for publication Feb. 20, 1934; issued August 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 49.

[3] The manufacture of poi is described by Allen and Allen (*2*). After taro has been cooked, ground, and mixed with water, the product is known as fresh poi. Incubation and fermentation follow.

methylene blue method (*7*). The results, which are averages of triplicate determinations, are given in the following tabulation:

	Percent		Percent
Moisture	69. 3	Fat	0. 07
Starch (takadiastase)	27. 0	Crude fiber	. 39
Pentosans	1. 3	Ash	. 46
Reducing sugars	. 5	Phosphorus	. 057
Sucrose	. 03	Calcium	. 018
Proteins	. 31		

These data account for approximately 99.5 percent of the weight of poi used for analysis and, considering unavoidable variations in samples, the starch and reducing sugars found check well with those recorded in table 1 for 0-hour poi.

DETERMINATION OF STARCH AND REDUCING SUGARS PRESENT IN POI AT THE VARIOUS STAGES OF ITS FERMENTATION

Starch and reducing sugars were first determined together by acid hydrolysis of the starch. Then reducing sugars alone were determined by the colorimetric blood sugar method (*6*). The percentage of starch was obtained by difference. The chief difficulty encountered in this analysis lay in the mechanical separation of reducing sugars from starch. Centrifuging poi and water mixtures for 30 minutes at 1,500 revolutions per minute was substituted for filtering. The variations in the amounts of reducing sugars and starch present in poi during fermentation from the stage of fresh poi, 0 hour, to sour poi, 8 days, are shown in table 1.

TABLE 1.—*Reducing sugars and starch in poi at various stages of fermentation*

Age of poi at time analyzed	Reducing sugars	Starch	Age of poi at time analyzed	Reducing sugars	Starch	Age of poi at time analyzed	Reducing sugars	Starch
	Per-cent	*Per-cent*		*Per-cent*	*Per-cent*		*Per-cent*	*Per-cent*
0 hour	0. 53	25. 6	24 hours	0. 10	26. 0	5 days	0. 12	23. 4
6 hours	. 48	25. 8	2 days	. 11	25. 8	6 days	. 15	23. 2
12 hours	. 14	26. 0	3 days	. 14	24. 5	7 days	. 18	23. 2
18 hours	. 10	25. 8	4 days	. 12	23. 9	8 days	. 15	22. 2

Allen and Allen (*2*) state, from bacteriological evidence only, that there is little doubt that the fermentation of poi is a complex one and that the immediate evidence of fermentation, such as the evolution of carbon dioxide and an increase in acidity, indicate that carbohydrates are among the first substances to be attacked. The data in table 1 show a rapid decrease of sugars early in the fermentation. The fair degree of constancy of the percentage of reducing sugars from 12 hours to 8 days is consistent with the continuous diminution in starch content. The fairly constant values found for reducing sugars after the first 12 hours may be accounted for by a counterbalancing due to starch hydrolysis, or the constant values may indicate that all reducing sugars had been fermented and that the values found result from nonfermentable reducing substances present in the poi.

The decrease in starch content shown in table 1 was predicted by Allen and Allen (*2*) and is consistent with the anticipated behavior of the micro-organisms found by them. They state that there is reason to believe that during the period of acid production considerable starch is hydrolysed and thus converted into available energy. However, Miller (*8*), who studied the microbial fermentation of poi, failed to find any appreciable decrease in starch content. Allen and Allen (*2*), in commenting on Miller's data, state that since a pH value was not given for the fresh poi, the sample very probably was comparatively acid at the time of starch analysis and hydrolysis had already occurred.

DETERMINATION OF VOLATILE AND NONVOLATILE ACIDS PRESENT IN POI AT THE VARIOUS STAGES OF ITS FERMENTATION

The methods described in Official and Tentative Methods of Analysis (*3*) were used to determine volatile and nonvolatile acids. Fifty-gram samples of poi of the ages shown in table 2 were used. Each sample was mixed with 100 cc of water and centrifuged for 5 minutes at 1,500 revolutions per minute. The supernatant liquid was decanted, additional water placed in the centrifuge tubes, and the centrifuging and decanting repeated until 500 cc of a water extract of the poi sample was obtained. The extract was distilled by ordinary distillation with periodic checking of the distillate by titration and the addition of water to the distilling flask, as described in Official and Tentative Methods of Analysis (*3*). Volatile and nonvolatile acids were determined by titration with 0.2 normal sodium hydroxide solution. The results are given in table 2.

TABLE 2.—*Volatile and nonvolatile acids expressed as milligrams of hydrogen in 50-g samples*

Age of poi at time analyzed	Volatile acids	Nonvolatile acids	Age of poi at time analyzed	Volatile acids	Nonvolatile acids	Age of poi at time analyzed	Volatile acids	Nonvolatile acids
0 hour	1.00	0.93	1 day	1.60	1.40	7 days	3.56	4.41
2 hours	.87	.99	3 days	3.15	2.07	10 days	5.75	4.36

The micro-organisms found in Allens' bacteriological study to be present in poi are capable of inducing chemical changes which result in the formation of both volatile acids such as formic, acetic, propionic, and butyric, and nonvolatile acids such as lactic, oxalic, citric, malic, tartaric, and succinic. Those micro-organisms responsible for the volatile acids are known to be more active in the later stages of fermentation of poi than in the earlier stages.

IDENTIFICATION OF SPECIFIC SUBSTANCES PRESENT IN POI AT VARIOUS STAGES OF ITS FERMENTATION

The results of the complete analysis of poi, the study of reducing sugars and starch, the determination of volatile and nonvolatile acids, and the identification of micro-organisms through the bacteriological investigation made possible some definite predictions as to products resulting from the chemical changes induced by the micro-

organisms. Allen and Allen (*2*) state that the acid-producing bacteria of the *Streptococcus* and *Lactobacillus* species, the principal agents of fermentation, are able to produce not only large quantities of lactic acid but also moderate quantities of acetic, propionic, succinic, and formic acids, in addition to traces of acetone and alcohol. The species of yeasts, mycoderms, and oidia, present in much smaller numbers, could not account for the major chemical changes but could cause the formation of alcohol, carbon dioxide, and some acetic acid.

In the chemical study search was made for lactic, acetic, formic, propionic, butyric, malic, tartaric, succinic, citric, and oxalic acids, carbon dioxide, alcohol, acetone, and aldehyde at the stages from 0 hour to 10 days. Table 3 summarizes this study and is followed by a discussion of results.

TABLE 3.—*Results of experimentation to determine specific substances resulting from chemical changes induced by micro-organisms at various stages of poi fermentation* [1]

Substance found	0 hour	2 hours	1 day	3 days	5 days	7 days	10 days
Lactic acid	++	++	+++	+++	+++	+++	+++
Acetic acid	0	++	++	++	+++	+++	+++
Formic acid	0	++	++	++	++	++	++
Alcohol	0	+	+	+	+	+	+
Acetaldehyde	0	0	+	+	+	+	+
Carbon dioxide	+++	+++	+++	+++	+++	+++	+++

[1] 0 indicates the absence of the indicated substance, + that traces were found, ++ that the substance was present in small amounts, and +++ that appreciable quantities were present.

The treatment of poi samples and the methods used in testing for the various substances referred to in table 3 were briefly as follows: Alcoholic extracts (*1, v. 1*) of lactic, acetic, and formic acids, were obtained by triturating 100-g samples with 75 cc of 95-percent alcohol. The alcoholic extracts were filtered, diluted, made alkaline with excess of sodium carbonate, concentrated to small volumes, clarified with a decolorizing carbon, neutralized with hydrochloric acid, and boiled to remove carbon dioxide. These solutions were subjected to qualitative tests (*1, v. 1; 5*). To detect lactic, formic, and acetic acids, qualitative tests (*1, v. 1; 5*) were also applied to properly prepared samples, obtained from the distillation of volatile acids by the Dyer method (*5*).

The guaiacol, iodoform, aldehyde, and ferric chloride tests were used for lactic acid. Also, calcium lactate crystals were prepared from a sample of 900 g of fresh poi and identified by qualitative tests and by microscopic comparison with crystals of U. S. P. calcium lactate. For acetic acid, the concentrated sulphuric acid, ethyl acetate, and ferric chloride tests were applied. The mercuric chloride, silver nitrate, and ferric chloride tests were used for formic acid (*1, v. 1; 5*).

Tests for alcohol (*1, v. 1; 9*) and for acetaldehyde (*1, v. 1; 9*) were applied to samples of the distillates obtained in the determination of volatile acids and also to samples prepared as follows: One-hundred-gram samples of poi, at the fermentation stages indicated in table 3, were distilled with 150 cc of water, made slightly alkaline with 6 normal sodium hydroxide, and distilled over a water bath at a temperature of 50° C. and under pressure of 100 mm until 100 cc of distillate were

obtained. For alcohol, the ethyl acetate, ethyl benzoate, iodoform, and alkaline potassium permanganate tests were used, and for acetaldehyde, the Fehling, ammoniacal silver nitrate, aldehyde resin, and Nessler tests were applied.

Propionic acid, butyric acid, higher fatty acids (*5*), malic acid, tartaric acid, succinic acid, citric acid, oxalic acid (*1, v. 1*), and acetone (*1, v. 1*) were absent at all stages of fermentation. Tests for these substances were made both on alcoholic extracts of poi and on residues remaining from the distillation of volatile acids.

While it is known that a butyric ferment is present in poi, it was proved that lactic acid is not converted to butyric acid under ordinary conditions of fermentation either in the early stages of fermentation or in poi which has fermented as long as 15 days. However, butyric acid was isolated and identified (*1, v. 1; 5*) from a mixture of poi and calcium carbonate which had been allowed to stand for 15 days. No butyric acid was obtainable from poi in earlier stages of fermentation even in the presence of calcium carbonate. The well-known unpleasant odor and taste of very old poi may be accounted for by the formation of butyric acid as the acidity diminishes upon long standing.

SUMMARY AND CONCLUSIONS

The chemical investigation of the fermentations occurring in poi manufacture included a complete analysis of poi, the determination of reducing sugars, starches, volatile acids, and nonvolatile acids present at various stages of fermentation, and the identification of the products of fermentation. The analysis showed poi to be a starchy food consisting of about 69 percent water, 27 percent starch, and relatively small amounts of other substances. Reducing sugars decreased rapidly in the early stages of fermentation and remained fairly constant in amount from the stage of 12 hours to 8 days. Starches decreased from the second to the eighth day of fermentation.

The fermentation products identified were lactic acid, acetic acid, formic acid, alcohol, acetaldehyde, and carbon dioxide. These are consistent with the predictions made from the bacteriological study of the micro-organisms described by Allen and Allen (*2*). The results of the investigation show that the fermentations occurring in poi manufacture are due primarily to the action of micro-organisms on carbohydrates.

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RESULTS OF FEEDING SPROUTED OATS TO CORRECT STERILITY IN CATTLE AND SWINE[1]

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INTRODUCTION

The idea that sprouted oats might have value in restoring breeding power to cows temporarily sterile due to diet deficiencies probably had its origin in the work of Evans and Bishop [2] and others. These writers, working with rats, demonstrated the existence of a hitherto unrecognized vitamin which was later called vitamin E.

Preliminary experiments on the feeding of sprouted oats to temporarily sterile cows were initiated as early as 1923, and more elaborate experiments were started in 1925 at the Beltsville dairy farm of the Bureau of Dairy Industry of the United States Department of Agriculture.[3] This work was continued, and in 1930 the results of feeding sprouted oats to cows which were temporarily sterile and in which no pathological conditions could be found were given by Graves.[4] Miller and Graves [5] in reporting on the health records of the Beltsville herd give details on the methods of feeding sprouted oats and report that 57 females were directly benefited and 31 were not benefited, as judged by the number of services required to produce conception. In 27 cases, the cows conceived to a service just previous to the start of the oat feeding, and it is not definitely known whether such feeding was beneficial. There was no evidence that sprouted oats were helpful to the cows showing pathological changes in the genital organs.

Moore [6] of the Mississippi Agricultural Experiment Station fed sprouted oats to Jersey and Ayrshire heifers which had failed to breed. In several cases such oat feeding was helpful, but in others the results were negative.

EXPERIMENTS WITH CATTLE

EARLY OBSERVATIONS AT THE HAWAII STATION

The feeding of sprouted oats to heifers and cows that exhibited an abnormal breeding behavior was started in January 1927. These included animals which seemingly did not have the normal oestrum periods or, if the periods occurred normally, did not conceive when bred. Oats were germinated in trays and fed at the rate of 2 pounds (dry weight before germinating) per day until such time as the cows were thought to be pregnant. This plan was continued for about 3½ years.

[1] Received for publication Mar. 19, 1935; issued August 1935.

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Six of these oat-fed cows were animals that had failed to show any definite signs of being in heat after an average of 192 days after calving. Four of them exhibited heat periods and were successfully bred after an average of 13 days of oat feeding. A fifth cow was successfully bred in her second oestrum, which occurred 39 days after a 17-day oat-feeding period had been discontinued. The sixth cow was fed sprouted oats for 110 days, during which time she had two heat periods and was bred, but neither of these 2 services or the 6 that followed over a period of 1 year resulted in pregnancy.

One other cow had had 5 services, none of which resulted in pregnancy. This cow was then fed sprouted oats for 171 days, during which time there were 4 more services, none of which resulted in conception.

EXPERIMENTS WITH FEEDING OATS

As the early observations, while lacking controls, were in general rather favorable to sprouted oats as a feed, a more definite experiment was planned which began November 1, 1930. For this experiment, the following classes of cows were considered as having abnormal breeding behavior:

Class 1, heifers 24 months of age or older that seemingly show no heat periods. Such heifers were fed sprouted oats from the time they were 24 months old till 70 days after the last bull service.

Class 2, cows that had not come in heat within 4 months after calving. Such cows were fed sprouted oats after this 4-month period till 70 days after the last bull service.

Class 3, cows and heifers that had been bred once but had come in heat again. Such cows were fed sprouted oats till 70 days after the last service by the bull.

Class 4, the cows that had been the control groups in classes 1, 2, and 3 during the earlier part of the experiment and were still nonpregnant.

This plan included all animals in the herd except such as might be on some special test which would confuse the results. The first animal which fitted into one of the classes was fed sprouted oats as indicated; the second cow was not fed oats but was considered as a control animal; the third cow was fed oats; the fourth was not, etc. All the experimental animals received ample green feed daily.

Oats were germinated in trays and fed when the sprouts were from one-half to 2 inches long, at the rate of 2 pounds of oats (dry basis) per cow per day. For the most part, the cows ate the sprouted oats fairly well.

The tables show the results of feeding oats in the various classes. The reaction of each cow to the agglutination test for abortion is shown in all cases, the first character showing reaction at 1 to 25 dilution, the second at 1 to 50 dilution, and third at 1 to 100 dilution.

Table 1 gives the record of the cows in class 1.

TABLE 1.—*Record of individual cows in class 1*

Group	Cow no.	Agglutination test[1]	Period from 24 months of age to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
				Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
			Days	*Days*	*Days*	*Number*	*Number*	
Fed oats	103G	— — —	469	113	0	0	5	Yes.
	112H	P — —	2	2	69	0	1	Yes.
Control[2]	106H	+ — —	81	-------	-------	4	-------	Yes.
	121G	— — —	343	-------	-------	1	-------	No.

[1] Capital P means partial, plus sign (+) positive, minus sign (−) negative.
[2] Oats not fed.

Pregnancy did not occur in cow 103G till 353 days after sprouted-oats feeding had been stopped. Pregnancy occurred in cow 112H only 2 days after the feeding of oats was started. It is doubtful whether oats feeding was a determining factor in either case.

The data for the individual cows in class 2 that were continued in the herd long enough to get final calving data are shown in table 2.

TABLE 2.—*Record of individual cows in class 2*

Group	Cow no.	Agglutination test[1]	Date of last previous parturition	Period from calving to first observed heat period	Period from calving to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
						Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
				Days	*Days*	*Days*	*Days*	*Number*	*Number*	
Fed oats	97H	P — —	Aug. 11, 1930	191	369	227	0	0	4	Yes.
	100H	— — —	Nov. 7, 1930	165	299	91	0	0	2	Yes.
	86G	— — —	July 12, 1931	240	240	111	70	0	1	Yes.
	92H	— — —	July 31, 1931	141	426	99	0	0	4	Yes.
	49H	+ + +	Nov. 8, 1931	159	159	38	69	0	1	Yes.
	101G	— — —	Aug. 29, 1932	156	156	2	70	0	1	Yes.
	72H	— — —	Jan. 9, 1933	140	140	4	70	0	1	Yes.
	65H	— — —	Jan. 20, 1933	130	415	75	0	0	3	Yes.
	108H	— — —	Jan. 24, 1933	165	205	84	70	0	3	Yes.
Control[2]	91G	+ + +	June 2, 1930	175	479	-------	-------	5	-------	Yes.
	95H	— — —	Aug. 12, 1930	124	430	-------	-------	5	-------	Yes.
	99H	+ — —	Dec. 11, 1930	168	168	-------	-------	1	-------	Yes.
	62H	— — —	May 4, 1931	195	214	-------	-------	2	-------	Yes.
	104H	— — —	July 20, 1931	139	139	-------	-------	1	-------	Yes.
	31H	+ — —	Sept. 12, 1931	133	133	-------	-------	1	-------	Yes.
	72H	+ — —	Oct. 16, 1931	178	178	-------	-------	1	-------	Yes.
	109H	P — —	Aug. 1, 1932	169	169	-------	-------	1	-------	Yes.
	113H	— — —	Aug. 27, 1932	-------	-------	-------	-------	0	-------	No.
	86G	— — —	Dec. 17, 1932	152	152	-------	-------	1	-------	Yes.

[1] Capital P means partial, plus sign (+) positive, minus sign (−) negative.
[2] Oats not fed.

All of the 9 oat-fed cows eventually produced calves; 9 of the 10 cows in the control group also produced calves. The 9 oat-fed cows were fed sprouted oats for an average of 81 days before pregnancy, and in addition 5 of them were fed oats for 70 days each after pregnancy. The plan was to feed all of them sprouted oats for 70 days after pregnancy, but in the case of 4 of the cows the final breeding period, which resulted in pregnancy, did not occur till 70 or more days after the previous service, which was assumed to have resulted in conception.

For the cows which produced calves, an average of 2.2 services were required per conception in both the oat-fed and the control groups.

Two of the cows, 72H and 86G, appear in both the oat-fed and control groups but for different gestation periods. This is possible since this experiment covered a period of nearly 4 years.

For the 9 oat-fed cows an average of 165 days occurred from the time of calving to the first observed heat period and 268 days to the service that finally resulted in pregnancy; for the 9 cows in the control group, the corresponding figures are 159 and 229 days, respectively.

The data for the cows in this class fail to show that feeding sprouted oats was of any value in bringing about conditions favorable to pregnancy.

In class 3, oat feeding was started after the first heat period that occurred after the one when the cow was first bred and continued for 70 days after the last heat period when the cow was bred. Seventy days provides time enough for three normal oestrum periods. If none occurred, it was assumed that the cow was pregnant and oat feeding was stopped. Actually, however, in 11 cases out of 27, heat periods did occur later after none had been observed in the 70-day oat-feeding period. In these cases after oat feeding had been stopped it was not started again, and oats were fed only previous to pregnancy, because the cow was believed to be pregnant when oat feeding was stopped. The results are shown in table 3.

Of 27 oat-fed cows and heifers in class 3, 20, or 74 percent, produced calves; of the 25 control animals, 18, or 72 percent, produced calves. The cows in the oat-fed group were fed oats an average of 115 days each, and for the 20 cows that produced calves, 3.4 services were required per conception. In the control group the 18 cows that produced calves required 4.2 services per conception.

Since a period of about 4 years elapsed during this experiment, a cow might appear twice in the same group or in both groups, for, as previously explained, every other cow exhibiting the same apparent breeding trouble, in the order in which it occurred, was fed oats. Thus, 2 cows appear twice each in the oat-fed group, 3 appear twice each in the control group, and 8 appear in both groups.

Of the oat-fed group, 7 cows were fed oats both before and after pregnancy; 12 were oats fed only before pregnancy and 8 only after pregnancy.

For the 18 oat-fed cows (heifers excluded) that produced calves, an average of 282 days elapsed between the last previous parturition and the service that finally resulted in pregnancy; for the 15 control cows (heifers excluded), an average of 252 days was required.

TABLE 3.—*Record of individual cows in class 3*

Group	Cow no.	Agglutination test [1]	Date of last previous parturition	Period from calving to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
					Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
				Days	*Days*	*Days*	*Number*	*Number*	
Fed oats	51G	— — —	Apr. 21, 1929	758	64	70	7	2	Yes.
	80G	— — —	July 19, 1929	472	70	0	2	0	No.
	64H	P — —	Feb. 9, 1930	272	0	70	4	0	Yes.
	98H	— — —			138	0	4	4	No.
	49H	+ + +	Feb. 12, 1930	325	0	70	2	0	Yes.
	45H	— — —	Mar. 30, 1930	1,112	449	0	3	12	No.
	74H	+ — —	May 29, 1930	214	19	78	2	1	Yes.
	31H	+ + —	June 10, 1930	185	0	70	2	0	Yes.
	89G	— — —	July 18, 1930	246	0	70	2	0	Yes.
	72H	P — —	Sept. 12, 1930	132	0	115	2	0	Yes.
	108H	— — —			0	70	2	0	Yes.
	96G	— — —	Nov. 27, 1930	263	70	0	2	1	Yes.
	42H	— — —	Jan. 9, 1931	173	20	70	2	1	Yes.
	77G	+ + +	Jan. 30, 1931	301	191	70	2	4	Yes.
	52H	— — —	May 3, 1931	414	70	0	4	0	Yes.
	101G	— — —	July 3, 1931	135	12	71	2	1	Yes.
	60H	— — —	Oct. 16, 1931	220	0	70	2	0	Yes.
	89G	— — —	Jan. 4, 1932	207	0	70	2	0	Yes.
	114G	— — —			69	0	2	1	No.
	102H	— — —	Apr. 25, 1932	258	69	0	2	6	No.
	122H	— — —			70	0	2	1	Yes.
	83H	— — —	July 11, 1932	252	17	70	2	1	Yes.
	111H	— — —	July 29, 1932	471	105	0	2	2	Yes.
	82H	— — —	Sept. 3, 1932	225	87	71	2	3	Yes.
	105H	— — —	Sept. 21, 1932	290	86	0	2	2	Yes.
	85H	— — —	Oct. 14, 1932	535	287	0	2	9	No.
	42H	— — —	Mar. 30, 1933	385	117	0	2	5	No.
Control [2]	60H	— — —	Dec. 15, 1929	392	------	------	5	------	Yes.
	82H	— — —	May 31, 1930	184	------	------	3	------	Yes.
	32G	— — —	June 29, 1930	367	------	------	4	------	No.
	68H	+ + +	June 30, 1930	194	------	------	5	------	Yes.
	94G	— — —	July 9, 1930	288	------	------	5	------	Yes.
	105H	— — —			------	------	9	------	Yes.
	65H	— — —	Sept. 23, 1930	199	------	------	4	------	Yes.
	107G	— — —			------	------	4	------	Yes.
	83H	P — —	Nov. 19, 1930	324	------	------	6	------	Yes.
	59H	+ + P	Nov. 23, 1930	406	------	------	9	------	No.
	67G	+ + +	Dec. 26, 1930	199	------	------	4	------	Yes.
	109H	+ — —			------	------	3	------	Yes.
	85H	— — —	Jan. 14, 1931	367	------	------	8	------	Yes.
	88H	— — —	Mar. 10, 1931	112	------	------	2	------	Yes.
	71H	— — —	July 29, 1931	367	------	------	2	------	Yes.
	79H	P — —	Aug. 19, 1931	326	------	------	4	------	Yes.
	74H	P — —	Oct. 1, 1931	202	------	------	2	------	Yes.
	94G	— — —	Feb. 7, 1932	255	------	------	4	------	No.
	117G	— — —			------	------	2	------	No.
	110H	— — —	Feb. 28, 1932	240	------	------	2	------	No.
	51G	— — —	Mar. 7, 1932	311	------	------	3	------	No.
	77G	+ P —	Sept. 2, 1932	154	------	------	4	------	No.
	68H	— — —	Oct. 12, 1932	235	------	------	4	------	Yes.
	112H	— — —	Dec. 17, 1932	123	------	------	2	------	Yes.
	74H	P — —	Jan. 28, 1933	265	------	------	3	------	Yes.

[1] Capital P means partial, plus sign (+) positive, minus sign (—) negative.
[2] Oats not fed.

Examinations for abnormal conditions in the reproductive organs of the cows were made in only a few cases.

Except that the number of services required per conception were slightly lower in the case of the oat-fed cows, there is little else to suggest that feeding oats was helpful to the cows in this class.

During the latter part of the experiment, the cows that had formed the control groups of the first three classes were designated as class 4 and were fed sprouted oats to note their effect on animals that had

failed to conceive normally over a long period. Class 4 included all controls in the dairy that had not had a heat period or had had a reoccurrence of a heat period 7 months or later after calving, and heifers that had not yet calved, that had not had a heat period by 27 months of age, or had had a reoccurrence of a heat period after that age. It was believed that if such animals should become pregnant after feeding sprouted oats, it would definitely indicate that sprouted oats had value in correcting sterility in dairy cattle.

In the case of 3 of these cows, 2 of which had had 3 previous services and 1 had had 2, the service preceding the beginning of oat feeding by 2, 3, and 4 days, respectively, resulted in pregnancy, indicating a value in possibly preventing reabsorption of the fetus.

One sterile heifer that had had 3 services previous to feeding oats was fed sprouted oats for 68 days, during which time no symptoms of being in heat were observed; but after oat feeding had been stopped, there were 4 more heat periods at each of which she was bred but pregnancy did not result. An examination by a veterinarian failed to show any pathological conditions in the reproductive organs of this heifer.

Three other cows in this group seemingly were not benefited by feeding oats, but for reasons of economy it was necessary to dispose of them.

EXPERIMENTS WITH SWINE

EARLY OBSERVATION TESTS

The feeding of sprouted oats to sows was started early in 1928, about 1 year after observation tests along this line were started with cows. Sprouted oats were fed at the rate of 1 pound (dry basis) per sow per day to sows that had an abnormal breeding schedule until the sows were believed to be pregnant. Eighteen such sows were oat-fed, 5 of which later proved to be pregnant by a service which preceded the oat-feeding period. Of the other 13 sows, 11 produced litters, and with 7 of these sows there is some indication that the feeding of sprouted oats was helpful. Oats were fed for an average of 106 days. The other 4 sows that produced litters did not conceive till an average of 134 days after oat feeding had been discontinued. The two that did not produce litters were fed sprouted oats for 202 and 195 days, respectively, but to no avail.

The abnormal or irregular breeding schedule mentioned above of the 9 sows consisted in their not having any observed heat periods and, of the other 4 sows, in having repeated heat periods after having been bred once, or in some cases, several times.

LATER EXPERIMENTS

The observation tests seemed slightly favorable to feeding sprouted oats, so a more definite experiment was planned November 1, 1930, which provided for control as well as oat-fed animals and also classes for different types of breeding irregularities.

Class 1, gilts 10 months or older that seemingly did not come in heat. Such gilts were fed sprouted oats from the time they were 10 months old till 50 days after the boar service which seemed to have resulted in pregnancy.

Class 2, sows that had not been observed in heat 4 months after farrowing. Such sows were fed sprouted oats till 50 days after the last service by the boar.

Class 3, sows that had been bred once but came in heat again. Such sows were fed sprouted oats beginning at the first heat period following the one when the sow was bred, and oat feeding was continued till 50 days after the last boar service.

Class 4, sows in the control groups of classes 1, 2, and 3, that had failed to breed after a long period.

The first animal which fitted into one of the three classes was fed sprouted oats, the second was not fed oats but was considered as a control, the third sow was oat-fed, etc. Occasionally, for various reasons, some deviation from this plan was necessary. Both the oat-fed and the control animals were generally fed at least 1 pound of green alfalfa or other green feed per animal daily.

Oats were germinated in trays and fed when the sprouts were from one-half to 2 inches long at the rate of 1 pound (dry basis) per sow per day.

The results in class 3 are shown in table 4. During the period of this experiment, November 1, 1930, to the end of 1934, all of the gilts had heat periods by the time they were 10 months old, so there were no animals that fitted into class 1. Only 1 sow in class 2 was fed oats, and she had a heat period and was successfully bred 2 days after oat feeding was started. In the control group 3 sows not fed oats were successfully bred at the first observed heat period 141, 143, and 193 days, respectively, after the previous farrowing date.

TABLE 4.—*Record of individual sows in class 3*

Group	Sow no.	Date of last previous parturition	Period from farrowing to service that resulted in pregnancy	Period oats were fed		Boar services		Litter produced
				Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
			Days	*Days*	*Days*	*Number*	*Number*	
Fed oats	84T	Aug. 31, 1929		121	0	3	4	No.
	103T			20	50	3	1	Yes.
	105B	July 7, 1931	131	0	50	2	0	Yes.
	110T	Nov. 28, 1931	153	17	50	2	1	Yes.
	113B			16	50	2	1	Yes.
	114B			0	47	2	0	Yes.
	111T	June 9, 1932	147	0	50	2	0	Yes.
	114B	Nov. 27, 1932	158	32	50	2	1	Yes.
	121B			0	50	2	0	Yes.
	123B			39	51	2	2	Yes.
	124T	Sept. 5, 1933	173	73	50	2	3	Yes.
	125T			82	0	5		No.
	114B	July 29, 1933	381	71	0	2	5	Yes.
	121B	May 10, 1934	138	0	58	2	0	Yes.
Control [1]	87T	Feb. 24, 1931				2		No.
	103T	Dec. 1, 1931	120			3		Yes.
	112B					2		Yes.
	115B					2		No.
	105B	Oct. 10, 1932				4		No.
	124T					2		Yes.
	106B	Mar. 30, 1933	195			6		Yes.
	118T	Mar. 14, 1933	156			4		Yes.
	109T	July 13, 1933	112			3		Yes.
	23T					5		No.
	118T	Dec. 9, 1933	254			6		Yes.
	123B	do	135			2		Yes.
	109T	Mar. 3, 1934	197			3		Yes.

[1] Oats not fed.

Of the 14 oat-fed sows in class 3, 12, or 86 percent, produced litters; of the 13 controls, 9, or 69 percent, produced litters. The sows in the former group were fed oats an average of 73 days each, and for the

12 sows that produced litters, an average of 3.25 services were required per conception. In the control group, the 9 sows that produced litters required 3.44 services per conception.

During the period of the experiment, 1 sow appears twice and 1 sow three times in the oat-fed group. Two sows each appear twice in the control group, and four different sows are found in both groups. This is possible since a period of about 4 years elapsed during the time of the experiment.

Of the oat-fed group, 6 sows were fed sprouted oats both before and after pregnancy; 3 were oat-fed only before, and 5 only after pregnancy.

For the sows that had previous litters, the average number of days that elapsed between the previous farrowing date and the service that finally resulted in pregnancy was 183 and 167 days, respectively, for the oat-fed and the control groups.

While the farrowing percentage is a little higher with the oat-fed sows, there is little else in these data to indicate that the feeding of sprouted oats was particularly helpful in correcting sterility or reducing the time required for conception to occur.

Class 4, which was started in January 1932, included all sows in the control groups that had not had an observed heat period or had a reoccurrence of a heat period 7 months or later after farrowing. These sows were fed sprouted oats till 50 days after the occurrence of the last heat period.

Of 5 sows that had failed to become pregnant while in the control groups and hence were put in class 4, 3 produced litters, the average period of feeding oats being 51 days. Two others that were fed oats for 74 and 132 days, respectively, failed to conceive and were finally sold.

SUMMARY AND CONCLUSIONS

It has been suggested that sprouted oats may contain a vitamin or other substance which perhaps because it prevents reabsorption of the embryo or other beneficial action, causes animals that are temporarily sterile to again become pregnant.

Early experiments with feeding sprouted oats to such animals gave rather favorable results. For lack of controls, however, definite conclusions were not possible and an experiment was planned in which every other breeding animal exhibiting a certain abnormal breeding behavior was fed sprouted oats and the alternating animals were placed in control groups.

Of 38 cows with an irregular breeding behavior, to which sprouted oats were fed, 31, or 82 percent, produced calves; of 37 cows with the same irregular breeding behavior that were put in the control group, 28, or 76 percent, produced calves.

Although in the case of some cows the feeding of sprouted oats seemed definitely helpful, equally good results were secured where oats were not fed. In general, this experiment does not demonstrate any definite value of sprouted oats for correcting sterility in cows.

Of 15 oat-fed sows, 13, or 87 percent, produced litters; of 16 control animals, 12, or 75 percent, produced litters. In addition, 3 of 5 sows that were fed sprouted oats after having failed to conceive for a long period in the control groups finally produced litters. While there is some indication here that feeding sprouted oats was of some

value when fed to shy breeders, the evidence is by no means conclusive.

All cows in the university herd receive ample green feed daily, and all sows in the university piggery, including both oat-fed and control groups were generally fed at least 1 pound of green alfalfa or other green feed per sow per day. If sprouted oats contain some substance resulting in beneficial action to shy breeders, it is possible that the same substance may be found in other green feeds. The feeding of sprouted oats to shy breeders might show more definite results if other green feeds were withheld from the experimental animals.

STUDY OF THE REMOVAL OF SPRAY RESIDUES FROM APPLES [1]

By DONALD E. H. FREAR, *instructor in agricultural and biological chemistry*, and H. N. WORTHLEY, *associate professor of economic entomology, Pennsylvania Agricultural Experiment Station* [2]

INTRODUCTION

The problem of the removal of spray residues from apples is of comparatively recent origin in Pennsylvania, where climatic conditions and a relatively light infestation of the codling moth until 1929 combined to permit the production of clean fruit with few spray applications. Since that time, however, the codling moth populations in the commercial apple-growing centers have increased to such an extent that heavier and more frequent applications of arsenicals are now necessary. A schedule calling for five cover sprays in 1932 caused some crops to show residues of arsenic in excess of the tolerance. In 1933 restrictions were placed on lead, regarding the removal of which little was known. During the 1933 season, therefore, spraying was reduced to a minimum, with the result that excessive residues were avoided at the cost of an alarming increase in codling moth damage. It became clearly evident that intensive spraying would be necessary in 1934, and that the apple crop would doubtless require washing to meet residue tolerances.

While the fundamental facts concerning the removal of spray residues from apples are rather well understood, the fact that conditions peculiar to a locality do exist makes generalization impossible. In addition, the recent developments in methods for the accurate determination of small amounts of lead make possible a more thorough understanding of the behavior of this element under washing conditions.

During the season of 1934 a program of research in methods of apple washing was instituted, the results of which are reported in this paper.

The work was planned to give information on the method best suited for the removal of spray residues from apples grown under Pennsylvania conditions. A number of factors of climate and locality are obviously variable in any study of this kind. The extended investigations on spray-residue removal conducted at other institutions, therefore, are valuable only insofar as they can be applied to local conditions; hence some of these studies repeat, for Pennsylvania, work which has been done elsewhere. It will be noted later that certain conclusions and recommendations based on investigations in other localities do not apply here.

[1] Received for publication Mar. 19, 1935; issued August 1935. Technical paper no. 682.

[2] The authors express their appreciation to Merrill Wood for technical assistance and to F. N. Fagan and A. W. Clyde for the use of the facilities of the Departments of Horticulture and Agricultural Engineering in this work. The authors also are indebted to S. W. Frost for the use of laboratory space, and to the fruit growers who contributed apples for experimental purposes.

EXPERIMENTAL CONDITIONS AND PROCEDURE

Part of the work was done in a laboratory set up in the largest commercial apple-growing section of Pennsylvania, in the south-central part of the State. The samples, for the most part, were collected with the cooperation of the growers of that section, but some were collected from the college orchard from experimentally sprayed trees; the work on these samples was conducted in the laboratories at State College, Pa. Over 500 samples were experimentally washed.

The varieties of apples were as follows: York Imperial, Stayman Winesap, Grimes Golden, Jonathan, Rome Beauty, Smokehouse, Delicious, Ben Davis, Stark, Hubbardston, and Yellow Newtown. The latter five varieties were represented by only a few samples.

The mean temperature for the south-central section of the State was slightly above normal during the season of 1934 except during August. The rainfall records taken at Arendtsville indicated a total precipitation for the summer months as follows: June, 6.04 inches; July, 2.51 inches; August, 5.96 inches; September, 14.09 inches. On each of 6 days during September, 1 inch or more of rain fell; the rainfall totaled 7.29 inches on 4 consecutive days, September 14 to 17, reaching nearly cloudburst proportions with 3.48 inches on September 17. The figures indicate nearly normal rainfall during June and July, when most of the codling-moth spraying was done, and a large excess in August and September, most of this coming in heavy showers during the latter month. Thus in 1934 spray applications were subjected to an average amount of weathering during the spraying period and to a great excess of rainfall between the time of the last application and the date of harvest, especially for the late varieties. It is questionable that heavy concentrated downpours of rain are as effective in removing spray residues as are more gentle rains scattered throughout the growth period of the fruit. Samples analyzed both before and after the heavy September rains showed surprisingly little reduction in spray residues from this cause. It would appear probable, therefore, that the distribution of rainfall rather than the total quantity has the greater effect on residue levels at the time of harvest.

Samples were taken by the authors, usually from the lower limbs of trees in the orchards. A sufficient number of trees were sampled to give a representative lot of apples of as nearly the minimum packed size as could be determined at the time of picking. An attempt was made to select apples of a fairly uniform size. It is well known that the smaller apples from the lower limbs of the trees usually carry the greatest amount of spray residue. When necessary, apples were taken from picking crates, but the same care was exercised in selecting for size and uniformity. Most samples were washed within 10 days after picking.

In the analysis of apple samples for lead, the method of Frear and Haley (*3*) [3] was used, while arsenic was determined by the Gutzeit method, as described in the official methods (*1, pp. 306–309*). Both of these methods have been found satisfactory, although the accuracy of the lead determination is apparently greater than that of the arsenic estimation. The figures for the latter element are the mean of at least two determinations. The relative accuracy of the lead and

[3] Reference is made by number (italic) to Literature Cited, p. 73.

arsenic methods, as well as the accuracy of sampling of apples, will be discussed elsewhere.

The apple washers used were of two types: One was a flotation type, similar to the washer designed by Jennings,[4] but with structural modifications. These modifications did not affect the operation of the machine, the principle of operation of which was similar to several other types of flotation washers (*2*, *4*). The other washer was a commercial underbrush type of the latest model, in which the washing solution is violently agitated and thrown over the apples as they advance over roller brushes. This machine was fitted with a roller drier, while the flotation machine was not equipped with a drying apparatus.

EFFECT OF VARIOUS FACTORS ON EFFICIENCY OF RESIDUE REMOVAL

The experimental work was divided into a study of the effects of the following factors on the efficiency of apple washing: Type of washer, type of washing solution, wetting agents, the use of heated solutions, spraying materials used, time of application, and variety of fruit.

EFFECT OF TYPE OF WASHER

In this study only two types of washer were available for most of the comparisons, the flotation and the underbrush type.

Table 1 gives the data secured by washing two varieties of apples sprayed with three different spray mixtures in flotation and brush machines. While individual samples showed considerable variation, a statistical analysis of the figures presented showed the underbrush washer to be significantly more efficient than the flotation washer in removing lead; the underbrush machine also was more efficient in the removal of arsenic, but the difference was not so pronounced.

The six samples identified in this and subsequent tables as 1, 2, and 3, are of both the York Imperial and Stayman Winesap varieties. They were secured in the college orchard and were treated as follows: Sample 1 received 6 cover sprays of 3 pounds of lead arsenate plus 8 quarts of lime-sulphur solution in each 100 gallons; sample 2 received 6 cover sprays of the same material, to which was added 2 pounds of skim-milk powder per 100 gallons; sample 3 received 6 cover sprays each containing 3 pounds of lead arsenate, 5 pounds of flotation sulphur and 1 quart of fish oil (cold-pressed menhaden, with less than 2 percent free fatty acids) in each 100 gallons of spray.

The underbrush washer was also more effective than the flotation washer when the hydrochloric acid solution was used with a wetting agent, as shown in table 2. In these studies the period of exposure to the acid was 1 minute in the flotation washer, and 40 seconds in the underbrush machine.

EFFECT OF DIFFERENT WASHING SOLUTIONS

Several washing solutions were tested for their efficiency in removing arsenic and lead residues from apples.

[4] JENNINGS, B. A. THE CORNELL APPLE WASHER. Cornell Mimeograph Bull. 279, 14 pp. 1934.

TABLE 1.—*Comparative efficiency of lead and arsenic spray residue removal from York Imperial and Stayman Winesap apples by means of flotation and underbrush washers, various percentages of hydrochloric acid being used*

YORK IMPERIAL, LEAD

Percentage of hydrochloric acid in wash	Temperature (° F.)	Grain of lead or arsenic per pound of fruit in—					
		Sample 1		Sample 2		Sample 3	
		Flotation-washed	Brush-washed	Flotation-washed	Brush-washed	Flotation-washed	Brush-washed
Before washing		0.044	0.044	0.054	0.054	0.060	0.060
1	60	.016	.014	.016	.018	.025	.020
2	60	.020	.016	.012	.009	.020	.021
1	100	.019	.009	.015	.010	.016	.013
2	100	.013	.016	.012	.009	.021	.021
Average		.017	.014	.014	.012	.021	.019

STAYMAN WINESAP, LEAD

Before washing		0.049	0.049	0.075	0.075	0.082	0.082
1	60	.016	.015	.018	.012	.022	.018
2	60	.016	.011	.010	.018	.020	.018
1	100	.021	.008	.020	.014	.013	.012
2	100	.012	.013	.014	.016	.016	.014
Average		.016	.012	.016	.015	.018	.016

YORK IMPERIAL, ARSENIC TRIOXIDE

Before washing		0.015	0.015	0.022	0.022	0.020	0.020
1	60	.004	.003	.006	.006	.010	.006
2	60	.006	.003	.004	.003	.006	.005
1	100	.004	.003	.006	.003	.004	.005
2	100	.003	.003	.003	.002	.005	.004
Average		.004	.003	.005	.004	.006	.005

STAYMAN WINESAP, ARSENIC TRIOXIDE

Before washing		0.015	0.015	0.022	0.022	0.017	0.017
1	60	.003	.005	.004	.004	.007	.007
2	60	.004	.002	.003	.003	.005	.005
1	100	.003	.004	.004	.006	.005	.005
2	100	.002	.001	.003	.004	.005	.004
Average		.003	.003	.004	.004	.006	.005

SODIUM SILICATE

Since sodium silicate has been used with considerable success in the apple-growing sections of the Pacific coast, an attempt was made to determine its practicability under local conditions. It was found that a cold solution of sodium silicate did not remove any appreciable amount of residue when used at a concentration of 80 pounds per 100 gallons, and even when heated to 100° F. was not so effective as hydrochloric acid. It may be mentioned at this point that few Pennsylvania growers have found it necessary to use oil sprays to any extent, and the beneficial effect of an alkaline wash is observed most readily when heavy oil applications have been made to the fruit.

TABLE 2.—*Effect of wetting agents on the efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples by means of flotation and brush washers, a 2-percent hydrochloric acid wash solution being used*

Sample	Grains per pound of fruit of—					
	Lead			Arsenic trioxide		
	Original load	After 2 percent HCl washing	After 2 percent HCl washing + wetting agent B [1]	Original load	After 2 percent HCl washing	After 2 percent HCl washing + wetting agent B [1]
FLOTATION WASHER, 1 MINUTE						
York Imperial 1	0.044	0.020	----------	0.015	0.006	0.002
Stayman Winesap 1	.049	.016	0.009	.015	.004	.001
York Imperial 2	.054	.012	.011	.022	.004	.001
Stayman Winesap 2	.075	.010	.016	.022	.003	.003
York Imperial 3	.060	.020	.015	.020	.006	.004
Stayman Winesap 3	.082	.020	.016	.017	.005	.004
UNDERBRUSH WASHER, 40 SECONDS						
York Imperial 1	0.044	0.009	0.007	0.015	0.003	0.001
Stayman Winesap 1	.049	.011	.013	.015	.002	.002
York Imperial 2	.054	.010	.009	.022	.003	.003
Stayman Winesap 2	.075	.018	.018	.022	.003	.003
York Imperial 3	.060	.016	.008	.020	.005	.002
Stayman Winesap 3	.082	.018	.012	.017	.005	.003

[1] A commercial defoaming agent was added to the solutions containing the wetting agents in both types of washers. As given in this and subsequent tables, wetting agent A was Areskap, 1 gallon per 100 gallons, and wetting agent B was Vatsol, 8 pounds per 100 gallons, plus 2 quarts of Degras (Antifoam no. 16).

SODIUM CARBONATE AND SOAP

A commercial preparation composed of sodium carbonate and a coconut-oil soap was tried in various concentrations, but was not considered effective enough to warrant extended investigation.

HYDROCHLORIC ACID

Since dilute solutions of hydrochloric acid are the most widely used washing liquids, a large number of washings were made with this material. It was found that among the solutions tested it was the most effective in reducing the amount of the residues. The concentration necessary to use on various varieties showing different levels of residue was studied in some detail.

A large number of lots of apples of different varieties under various spray treatments were run through the flotation washer at room temperature, and the quantity of lead and arsenic removed by using hydrochloric acid at three concentrations was determined. The time of immersion in the washer was 1 minute; and the concentrations are given in percentage by weight of hydrochloric acid. Table 3 shows the relative effectiveness of the three concentrations in removing lead and arsenic. The results are expressed in percentage of residue (lead or arsenic trioxide) remaining on the fruit.

Maximum removal was effected by the highest concentration, and only at this concentration was the removal of arsenic proportionately as great as that of lead. This important consideration has apparently

not been mentioned in previous studies. Based on an average of all samples from various sources, the greatest efficiency of removal of both lead and arsenic was apparently at an acid concentration of approximately 2 percent by weight, when the period of immersion was 1 minute, in a flotation washer. These generalized relationships may be changed by various factors, as will be shown later.

TABLE 3.—*Percentage of original arsenic and lead spray residue remaining on apples*[1] *after 1-minute exposure in flotation washer, various percentages of hydrochloric acid being used*

Hydrochloric acid strength	Number of samples	Original residue remaining on fruit after washing	
		Lead	Arsenic trioxide
		Percent	*Percent*
0.5	10	44.5	61.5
1.0	74	33.1	37.9
2.0	17	29.5	26.8

[1] See p. 62 for varieties represented in this table.

Hydrochloric Acid and Salt

The addition of salt to solutions of hydrochloric acid has been advocated by several workers (*2*, *7*). Overley et al. (*6*), however, indicate questionable results at temperatures less than 110° F. An investigation of the possible usefulness of salt was undertaken. The figures in table 4 indicate that when small quantities of salt (1 percent) are added to dilute acid in a flotation washer the efficiency of the acid is not appreciably increased. When larger quantities of salt are added there is a decided decrease in the amount of lead removed, while the amount of arsenic removed is very slightly increased.

TABLE 4.—*Effect of added sodium chloride on the efficiency of lead and arsenic spray-residue removal from apples with hydrochloric acid wash solution*

Composition of wash solution	Number of samples	Average lead residue remaining on fruit after washing with—		Average arsenic trioxide residue remaining on fruit after washing with—	
		Acid alone	Acid +NaCl	Acid alone	Acid +NaCl
		Percent	*Percent*	*Percent*	*Percent*
1 percent HCl containing 8 pounds NaCl per 100 gallons	2	26	26	50	45
1 percent HCl containing 50 pounds NaCl per 100 gallons	3	28	35	25	21
1 percent HCl containing 100 pounds NaCl per 100 gallons	3	28	35	25	21
2 percent HCl containing 100 pounds NaCl per 100 gallons	3	23	27	28	26

Mixed Acids

A mixture of 1 percent of hydrochloric acid and 0.5 percent of nitric acid was tried as a residue-removing solution. No apparent benefit was secured by the addition of nitric acid.

EFFECT OF WETTING AGENTS

The work of McLean and Weber in 1931 (*5*) indicated that the use of a wetting or foaming agent increased the effectiveness of washing in the removal of spray residues. Several commercial products have appeared on the market, and a test of their efficiency under Pennsylvania conditions was considered desirable. In using these various products, the recommendations of the manufacturer regarding the quantity to be used were followed, when such recommendations were given; otherwise a 1-percent solution by weight of the wetting agent was used. Table 5 gives typical results obtained. The data given in this table indicate that at least for the conditions of this test, these two wetting agents (the commercial preparations available to the apple industry) showed no consistent benefit.

TABLE 5.—*Effect of 2 commercial wetting agents on the efficiency of lead and arsenic spray-residue removal from several varieties of apples, 1- and 2-percent hydrochloric acid wash solutions being used*

Variety of apples	Grains per pound of fruit of—					
	Lead			Arsenic trioxide		
	Original load	After HCl washing	After HCl washing + wetting agent	Original lond	After HCl washing	After HCl washing + wetting agent
1 PERCENT HCl AND WETTING AGENT A[1]						
Jonathan	0.140	0.033	0.034	0.024	0.013	0.012
Grimes Golden	.080	.026	.018	.023	.013	.011
Smokehouse	.070	.020	.018	.017	.008	.009
Hubbardston	.048	.028	.024	.018	.012	.009
1 PERCENT HCl AND WETTING AGENT B						
Jonathan	0.140	0.033	[2] 0.029	0.024	0.013	[2] 0.017
Do	.098	.033	.036	.025	.013	.013
Grimes Golden	.083	.026	.030	.030	.011	.015
Smokehouse	.054	.014	.015	.028	.011	.006
Hubbardston	.084	.042	.043	.038	.021	.026
2 PERCENT HCl AND WETTING AGENT A						
Jonathan	0.140	0.026	0.032	0.024	0.006	0.018
Do	.088	.023	.022	.043	.008	.009
Grimes Golden	.084	.020	.025	.038	.007	.011
Do	.073	.020	.017	.025	.007	.011
York Imperial	.122	.030	.022	.048	.009	.013
Stayman Winesap	.038	.008	.008	.013	.003	.004
2 PERCENT HCl AND WETTING AGENT B						
Jonathan	0.088	0.023	0.024	0.043	0.008	0.011
Grimes Golden	.084	.024	.024	.032	.011	.010
Do	.073	.020	.024	.025	.007	.018
York Imperial	.124	.037	.031	.038	.008	.020
Stayman Winesap	.038	.008	.009	.013	.003	.006

[1] Wetting agent used with 1 percent NaCl according to manufacturer's recommendations.
[2] Double manufacturer's recommendations.

A further study of the effectiveness of wetting agents was conducted on apple samples dipped in a smaller amount of washing solution, closely simulating in every way the treatment secured in a flotation washer. These results are shown in table 6. The apples used in this experiment received six cover sprays each containing 3 pounds of lead arsenate, 5 pounds of flotation sulphur, and 1 quart of fish oil in each 100 gallons of spray. There was no significant increase in removal of

the residue when the wetting agents were used in addition to the acid at room temperature, and a slight but consistent apparent increase in the percentage removal when wetting agent A was used with acid solution at 100° F.

TABLE 6.—*Effect of commercial wetting agents on the efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples, when hand-dipped in 1 percent hydrochloric acid wash solution at different temperatures*

Washing treatment [1]	Grain of residue per pound of fruit for—			
	York Imperial		Stayman winesap	
	Lead	*Arsenic trioxide*	*Lead*	*Arsenic trioxide*
Original load	0.095	0.038	0.076	0.025
1 percent HCl, 1 minute at 60° F	.024	.010	.014	.005
Plus wetting agent A	.018	.007	.015	.006
Plus wetting agent B	.024	.012	.012	.005
1 percent HCl, 2 minutes at 60° F	.015	.009	.012	.004
Plus wetting agent A	.013	.004	.009	.003
Plus wetting agent B	.011	.005	.011	.004
Plus wetting agent C	.020	.011	.012	.006
Plus wetting agent D	.016	.007	.024	.010
Plus wetting agent E	.015	.008	.020	.007
Plus wetting agent F		.004	.014	.004
1 percent HCl, 1 minute, at 100° F	.010	.005	.008	.003
Plus wetting agent A	.006	.001	.005	.001
Plus wetting agent B	.008	.003	.010	.007
Plus wetting agent C	.016	.006	.012	.006

[1] Wetting agents C, D, E, and F were experimental products of the Rubber Service Laboratories Co.

As a means of determining the possible effect of the type of washer on the efficiency of the wetting agent, samples of apples were washed both in the flotation washer and in the underbrush washer, a 2-percent hydrochloric acid solution and wetting agent B being used. These results have already been presented in table 2. It is apparent that there was no very decided advantage in the use of wetting agents in either washer, although the apples sprayed with fish oil (treatment 3) showed a slightly greater removal of lead when the wetting agent was used than when the acid alone was used. From all three spray residues the removal of arsenic was apparently slightly aided in some cases by the presence of the wetting agent.

On the whole, under the conditions of these tests, the use of wetting agents did not assure an increase in the amount of spray residues removed. However, some of the data showed that additional arsenic was removed when wetting agents were employed, thus indicating that in certain cases their use may be justified. The exact conditions under which wetting agents may be expected to yield benefits, however, were not revealed in this study.

EFFECT OF RAISING THE TEMPERATURE OF WASHING SOLUTION

The use of heated solutions in the washing of apples has been recommended by several investigators. Particularly when the residue present on the fruit is extremely large, the use of a warm washing solution theoretically should be one of the most economically effective methods of removal. Studies were made comparing the efficiency of hydrochloric acid wash at approximately 60° and 100° F. The complete data have been given in table 1. The percentages remain-

ing, given in table 7, indicate the average amount of residue remaining on the three samples of each variety studied.

TABLE 7.—*Comparative efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples by 1- and 2-percent hydrochloric acid wash solutions, used in flotation and underbrush washers at 60° and 100° F.*

Type of washer and variety of apple	Average residue remaining on fruit after washing with—							
	1 percent HCl at 60° F.		1 percent HCl at 100° F.		2 percent HCl at 60° F.		2 percent HCl at 100° F.	
	Lead	Arsenic trioxide	Lead	Arsenic trioxide	Lead	Arsenic trioxide	Lead	Arsenic trioxide
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Flotation:								
York Imperial	36	35	33	25	33	23	29	20
Stayman Winesap	28	26	22	23	23	20	21	19
Underbrush:								
York Imperial	33	26	20	18	29	20	29	16
Stayman Winesap	23	31	17	28	23	19	22	16

These figures show that under the conditions of this experiment the acid solutions when heated to 100° F. were definitely more effective in removing both arsenic and lead than were the corresponding acid solutions at 60°. The effectiveness of the 1-percent hydrochloric acid solution at 100° was equal to that of the 2-percent hydrochloric acid solution at 60°. The difference in ease of removal from York Imperial and Stayman Winesap, which will be discussed later, is brought out in this table.

The data on the washings in the brush machine shown in table 7 are not as consistent as might be desired. This is in part explained by the fact that with the facilities available it was not possible to maintain a constant temperature in the underbrush washer because of the violent agitation and the consequent rapid loss of heat. In the flotation washer the temperature was constant.

EFFECT OF SPRAY MATERIALS APPLIED

The ease of removal of any given spray deposit from the surface of an apple is undoubtedly affected greatly by the factors which determine the nature of this deposit. These may be complicated by a great number of variable conditions, viz., temperature, humidity, wind velocity—inasmuch as it affects speed of drying—rainfall, the chemical composition of the spray mixture applied, and the nature of the apple surface.

It was impossible, in this study, to determine the effects of all these factors, but some attention was given to the effect that the gross composition of the spray mixture may have upon the ease of removal. The data in tables 1 and 2 show the relative ease of removal of three basic spray mixtures on two varieties of apples.

The figures in table 1 have been summarized in table 8 for the three spray treatments. The percentages in the table are true averages of the four washing treatments given to fruit of each variety receiving the three different spray mixtures.

TABLE 8.—*Effect of the gross composition of the original spray used on the efficiency of arsenic and lead spray-residue removal from York Imperial and Stayman Winesap apples with hydrochloric acid wash solution used in flotation and underbrush washers*

Variety and sample no.	Original residue remaining on fruit after washing					
	Lead			Arsenic trioxide		
	Flotation machine	Underbrush machine	Average	Flotation machine	Underbrush machine	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
York Imperial, 1	39	31	35	29	20	24
York Imperial, 2	26	21	24	22	16	19
York Imperial, 3	37	31	33	31	25	28
Stayman Winesap, 1	33	24	29	20	20	20
Stayman Winesap 2	21	20	20	16	19	18
Stayman Winesap, 3	22	19	20	32	31	31

These data indicate that lead and arsenic behave differently under washing treatment. Both York Imperial and Stayman Winesap fruit sprayed with lead arsenate and lime-sulphur without sticker retained the least lead residue at harvest, while the inclusion of fish oil—with flotation sulphur rather than lime-sulphur—built up the greatest deposits (table 1).

Table 8 shows that the lead residue from treatment 2 which contained skim-milk powder, offered the least difficulty in cleaning. In treatment 1 (no sticker) and treatment 3 (fish oil) more of the lead was retained after washing. In treatment 3 lead was more completely removed than in treatment 1 on Stayman Winesap, but not on York Imperial.

TABLE 9.—*Effect of gross composition and method of application of the original spray used on the ease of lead and arsenic spray-residue removal from Grimes Golden apples with 1-percent hydrochloric acid wash solution used in flotation washers*

Spray treatment (6 sprays applied) [1]	Number of samples washed	Average residue remaining on fruit after washing	
		Lead	Arsenic trioxide
		Percent	*Percent*
Lead arsenate, 3 pounds, lime-sulphur, 2 gallons [1]	2	30	23
Lead arsenate, 3 pounds;[2] hydrated lime, 0.5 pounds, lime-sulphur, 2 gallons	2	39	41
Lead arsenate, 3 pounds; flotation sulphur, 5 pounds; fish oil, 1 quart	3	37	0
Lead arsenate, 3 pounds; skim-milk powder, 2 pounds; lethane 410, 8.5 fluid ounces;[2] lime-sulphur, 2 gallons	4	37	41
Lead arsenate, 3 pounds; skim-milk powder, 2 pounds; Black Leaf 40, 1 pint;[2] lime-sulphur, 2 gallons; hydrated lime, 0.5 pounds [4]	6	32	38
Lead arsenate, 3 pounds; lime-sulphur, 2 gallons; pine-tar soap, 1 pint	1	38	33

[1] All quantities given are per 100 gallons of spray.
[2] Containing casein.
[3] In 2 sprays only, at oviposition peaks.
[4] In last cover spray only.

With respect to residues of arsenic, table 1 shows that treatment 1 built up the least, and treatment 2, containing skim-milk powder, the greatest deposits at harvest. As in the case of lead removal (table

8), treatment 2 cleaned most readily. Treatment 3, however, apparently formed an arsenic deposit considerably more difficult to remove than treatment 1, this being more pronounced on Stayman Winesap than on York Imperial.

Further study was made on Grimes Golden apples which had received six different spray treatments. These samples were washed in the flotation washer with 1-percent hydrochloric acid solution. The results are shown in table 9. Among the spray mixtures containing added stickers or spreaders there were apparently no significant differences in the ease of removal; all showed higher percentages of lead and arsenic not removed by the washing treatment than was the case with the mixture of lime-sulphur and lead arsenate.

It should be mentioned again that the amount of the original load on the fruit undoubtedly affects the efficiency of removal; higher loads being removed to a proportionately greater extent than lower loads. In the case of the data in table 9, this circumstance makes the statement in the last paragraph more significant, since in all cases the load on the fruit sprayed with lead arsenate and lime-sulphur mixture was lower than when modifiers were added.

Tables 8 and 9 suggest that the different added materials had a preferential effect in the ease of removal of arsenic and lead from the three apple varieties represented. For instance, more arsenic than lead was removed from fruit of all three varieites sprayed with the mixture containing no added sticker. This was also true for the mixture containing fish oil on Grimes Golden and York Imperial, but not on Stayman Winesap. Lead and arsenic were removed with almost equal facility from the mixture containing skim-milk powder on York Imperial and Stayman Winesap, whereas on Grimes Golden the arsenic was retained to a slightly greater extent.

EFFECT OF SPRAYING SCHEDULE

The number of applications and date of the last application in relation to date of harvest have a direct bearing on residue levels and consequently on the washing treatment necessary to bring these residues below the tolerance. It was impossible to give separate attention to this phase of the problem. However, it will be noted that the original loads reported in tables 5 and 6 were in many cases very high and were not in all cases reduced below tolerance by the washing treatments applied. The fruit sampled came from various orchards heavily infested with codling moth. The Smokehouse and Grimes Golden received 6 cover sprays ending in mid-July, while most of the other varieties were sprayed 7 to 9 times between the petal-fall application and mid-August. The York Imperial and Stayman Winesap in tables 1 and 2 received 6 applications ending in mid-July. Here arsenic removal was satisfactory with cold 1-percent hydrochloric acid, even where fish oil was used in the spray mixture. Lead removal, except where fish oil was used, appeared to be equally satisfactory in most cases, though 1 sample of York Imperial failed to react to 2-percent hydrochloric acid at 60° F. and 2 samples of Stayman Winesap to 1-percent hydrochloric acid at 100°.

EFFECT OF VARIETY OF APPLES

In the reported work on the effect of varietal differences on the efficiency of spray-residue removal, the information is neither definite nor complete. Overley, St. John, Overholser, and Groves (*6*) state that the Winesaps cleaned more readily than did Esopus Spitzenburg, Delicious, and Yellow Newtown. Since the Stayman Winesap apples retained more spray residue at harvest than some other varieties when subjected to the same spray treatment, it was considered likely that this variety might offer more difficulty in cleaning. This proved not to be the case, as shown by the results given in table 10.

TABLE 10.—*Effect of variety of apples on the ease of lead and arsenic spray-residue removal from the fruit with 1- and 2-percent hydrochloric acid wash solutions*

Percentage of acid and variety of apple	Number of samples	Average of original remaining on fruit after washing		Percentage of acid and variety of apple	Number of samples	Average of original residue remaining on fruit after washing	
		Lead	Arsenic trioxide			Lead	Arsenic trioxide
1-percent hydrochloric acid:		*Percent*	*Percent*	2-percent hydrochloric acid:		*Percent*	*Percent*
Grimes Golden	20	34	32	Grimes Golden	6	30	19
York Imperial	19	36	40	York Imperial	5	34	35
Stayman Winesap	17	27	38	Jonathan	4	28	27
Jonathan	6	30	39	Delicious	1	23	36
Rome Beauty	4	32	42	Stayman Winesap	1	21	23
Smokehouse	3	28	32				
Ben Davis	1	30	32				
Delicious	1	38	57				
Yellow Newtown	1	63	70				
Stark	1	42	33				
Hubbardston	1	50	55				

The factor of differential spray treatments is not considered in table 10, although it probably has considerable bearing on the removal of the residues by washing. In general, however, the type of application in these samples has been the same, lead arsenate 3 pounds to 100 gallons plus skim-milk spreader. The number of applications varied, however, from 3 to 8. No particular significance may be attached to those figures representing a single sample, but they are included in this table to indicate possible relationships which may exist between variety and ease of removal.

Table 10 shows that among those varieties of which sufficient numbers were examined to make interpretation possible, Smokehouse and Stayman Winesap cleaned most readily; Grimes Golden, Rome Beauty, and Jonathan came next while York Imperial offered considerably more difficulty.

Among the varieties represented by a single sample, Ben Davis appeared to be easy to clean, Stark offered moderate difficulty, and Yellow Newtown and Hubbardston were the two hardest to clean. Hubbardston in particular seemed to be, from other experiments, the most difficult to clean of all varieties tested.

SUMMARY AND CONCLUSIONS

This study attempted to determine the effects of various factors on the removal of arsenic and lead from apples under Pennsylvania conditions.

The removal of arsenic and lead were proportional to the concentration of hydrochloric acid, but a higher concentration of acid removed proportionately greater amounts of arsenic than lead. The concentration removing both of these elements in proportional quantities was approximately 2 percent by weight.

Wetting agents were of little value in increasing the efficiency of hydrochloric acid solutions in a flotation washer at room temperature, but a slight increase in the removal of arsenic resulted from their use in an underbrush machine. This increased efficiency was too small to be of any great importance, except possibly where fish oil was used in the spray mixture.

The variety of the apples washed was an important factor in the ease of residue removal by acid solutions. Listed in the order of increasing difficulty in residue removal, the varieties studied may be arranged tentatively as follows: Smokehouse, Stayman Winesap, Ben Davis, Grimes Golden, Rome Beauty, Jonathan, Stark, York Imperial, Delicious, while the few data avialable indicate that Yellow Newtown and Hubbardston are two varieties most difficult to clean.

The underbrush washer was slightly more effective in removing both arsenic and lead residues than the flotation washer, even though the time required for cleaning was less in the former.

Raising the temperature of the acid bath from 60° to 100° F. increased the efficiency, a 1-percent hydrochloric acid solution at 100° being approximately as effective as a 2-percent hydrochloric acid solution at 60°. The addition of salt, as recommended by several workers, did not increase the efficiency of acid solutions. Mixed hydrochloric acid and nitric acid solutions, sodium carbonate, and soap and sodium silicate were not so effective in lowering the residue on the fruit as dilute hydrochloric acid solutions.

Apparently the type of spray mixture applied had an effect on the ease of removal of arsenic and lead; this effect was not the same for both elements and was complicated by varietal differences. Fruit sprayed with mixtures containing a skim-milk spreader cleaned slightly more readily than did those sprayed with other combinations. The addition of fish oil to the spray mixture caused a deposit of residue more difficult to remove, but not so difficult as has been suggested in the literature.

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THE EFFECT OF NUTRITIVE STATE ON THE QUANTITY OF VITAMIN A PRESENT IN THE LEAVES OF COLEUS BLUMEI [1]

By E. S. HABER, *assistant professor of horticulture* and *research assistant in vegetable crops*, and PEARL P. SWANSON, *associate professor* and *research associate in foods and nutrition, Iowa Agricultural Experiment Station*

INTRODUCTION

Green leaves are known to be relatively rich in vitamin A and in its precursor, carotene. The factors involved in the synthesis of the vitamin have been studied from several angles. However, the writers have seen no data which show to what degree, if any, the nutritional state of the plant is reflected in the vitamin content of the leaf. The present investigation is concerned with this question.

The problem involves the production of distinct variations in the nutrition of the plant such as are manifested by differences in activity of growth, luxuriance of foliage, and depth of pigmentation. In order to obtain the necessary leaves for assay, two distinct methods of growing the experimental plants suggested themselves. Healthy and stunted plants can, of course, be grown very easily by planting cuttings in rich compost soil and in sand, respectively. The possibility of inducing two distinct planes of nutrition simultaneously in a single plant by subjecting its parts after division to different nutritive media was considered. With this method of control, the relative quantity of vitamin A in leaves obtained from branches of the same stock, the same age, but of different development could be determined. Such an experimental procedure, however, called for the assurance that no cross transfer of food nutrients occurs from one side of the plant to the other. Certain experiments described by Auchter (*1*) [2] point to the possibility of producing differentiated growth in a single plant. His data show that the foods manufactured on one side of a plant are used and stored in that portion of the plant or are translocated to the roots directly beneath. Magness (*5*) similarly postulates no cross transfer of food nutrients in woody plants like the apple, for he found that trees which were half defoliated formed very few fruit buds on the defoliated sides, whereas there was a normal development of fruit buds on the undefoliated parts.

On the basis of these reports, it seemed that the vitamin assay of leaves taken from laterals of a plant divided into two parts and so planted that each side received its nutrients from different media offered a unique means for enlarging the study of the problem of the relation of the nutritional state to the quantity of vitamin A synthesized in certain organs of the plant. The present investigation was therefore undertaken to demonstrate the relation of the nutritional state to the concentration of vitamin A in the leaves. The leaves

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[2] Reference is made by number (italic) to Literature Cited, p. 81.

were taken from undivided cuttings grown in soil, from those grown in sand, and from the two laterals of a plant whose stem and root system had been split so as to allow the planting of each side in soil and in sand, respectively.

EXPERIMENTAL PROCEDURE

PREPARATION OF PLANT MATERIAL

Many of our food plants have only one growing point, or the laterals arise from the axils of leaves alternately or in whorls, a system of development that makes impossible the objective of securing two branches of the same age. For the present study, therefore, a plant having an opposite leaf system with laterals developing simultaneously from the axil of opposite leaves was desired, whose growth could be effectively confined to two opposite laterals arising from the same node by the pinching back of all axillary branches that might appear. No food plant on hand met the necessary requirements. *Coleus blumei*, variety Golden Bedder, on account of its structure and habit of growth, was therefore chosen.

Three series of plants were prepared for the production of leaves for the assay. The plants composing each series were propagated by rooting stem cuttings in sand. After a sufficient root system had formed, each was potted in a small pot containing rich compost soil. After the plants were well rooted and growing, the main stem was pinched back to a strong node in order to secure branching at that point. When the root system was well developed, but before the plants became pot-bound, they were removed from the containers. All soil and organic matter were washed from the roots with a gentle stream of water. The plants composing series A were planted in clay pots 4 inches in diameter which contained rich compost soil; those in series B were grown in river-washed sand, known to be quite free from inorganic matter, while those in series C were plants which had developed two strong-growing laterals in the axils of opposite leaves at the same node. The stem of each plant in series C was split longitudinally in the middle for a distance of 1 to 1½ inches from the base of the stem toward the top. A few small roots were destroyed by this procedure. One part of the root system of the plant was then planted in the same mixture of soil that was used in series A, the other half in the sand. For this planting, two tin cans, size 3, that had been fastened together by means of a wire were employed. Small holes were punched in each can to allow drainage. The cans were nailed to a board in order to make the containers more rigid. The arrangement is shown in figure 1, *A*.

The plants of each series were then grown in the greenhouse until specific effects of the three treatments on the developing plants were evident. Four to six weeks were required. At the end of this time, considerable differential growth has occurred between the two opposite branches and the two root systems of the divided plants (fig. 1, *B*). The lateral on the same side of the plant as the root system which had been planted in sand remained small and stunted. The leaves were small and undersized but nearly normally pigmented. The lateral on the same side of the plant as the root system which had been planted in rich soil grew luxuriantly. It was 3 or 4 times as large as the opposite branch and carried many more well-developed leaves. The differ-

entiated growth showed that mineral and nitrogenous nutrients which were absorbed on the soil side of the plant were utilized and elaborated to a large extent on that side only. Apparently, the half of the plant growing in sand did not receive a sufficient quantity of nutrients from this medium to support normal growth.

THE ASSAY OF VITAMIN IN COLEUS LEAVES

Leaves of coleus taken from the three series of plants were offered to four different lots of rats as the sole source of vitamin A in a diet that had been rendered free of that factor. One lot, which will be

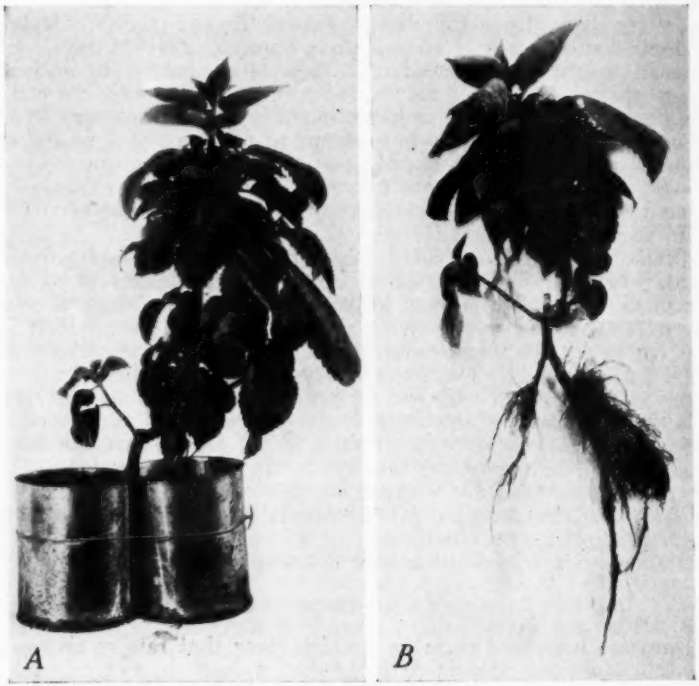

FIGURE 1.—*A*, Coleus planted with the root system divided so that one portion grew in sand and the other in rich soil, whereby differential growth was produced in two laterals of the same age; *B*, divided coleus plant removed from containers, showing extent of root system in sand and soil media after 3 months of growth.

referred to later as group A, was fed on leaves obtained from undivided plants grown in rich soil. The second lot (group B) was given the leaves taken from similar plants grown in sand, the third lot (group C) on leaves from the lateral of the divided plant grown in soil, and the fourth lot (group D) on leaves from the lateral grown in sand.

A modification of Sherman and Munsell's method (*6*) was used for the determination of the relative quantity of vitamin A in the four types of coleus leaves. The details of the method were carefully standardized in the nutrition laboratory in regard to the specific re-

action of the colony to the rations fed, respectively, to the stock rats and to the experimental animals.

The rats used in the study originated from the stock colony belonging to the nutrition laboratory of the foods and nutrition subsection. The colony was composed of Wistar rats inbred by brother and sister mating for 60 generations. The animals weighed approximately 47 g and were 28 days old when placed upon the experimental ration. The animals were caged individually and were fed the following vitamin-A-free diet ad libitum: casein (free from vitamin A), 18 percent; starch, 56 percent; Osborne and Mendel salt mixture, 4 percent; and Crisco, 22 percent. Five-tenths of a gram of yeast, one-fifth of which had been irradiated, was fed separately to each rat every day. Upon this dietary regime the animals were depleted of their bodily stores of vitamin A in approximately 21 days. Stationary weight for a period of 5 days, accompanied by incipient xerophthalmia, was used as the index of depletion. At the end of the depletion period, the average weight of the animals was 98.5 g. The rats were divided into four groups with litter mates, males, and females distributed as uniformly as possible in each group. Coleus leaves obtained from plants grown as described above were then offered to the members of each group as the only source of vitamin A in their food.

Preliminary trials indicated that 20 mg of coleus leaf grown in sand, when fed daily, furnished approximately 1 unit of vitamin A as defined by Sherman and Munsell (*6*). The four kinds of coleus leaves were therefore offered at this level. In sampling, each leaf was cut in segments and numbered according to the position of the segment in the leaf. On the first day, the first member of a group received segment 1; on the second day, segment 2, and so on. With this arrangement, as the experiment progressed, all rats received representative samples from all parts of the leaf, thus error due to unequal distribution of the vitamin in the leaf was avoided. The segments were carefully weighed on a torsion balance. A double portion was offered on Saturday. Special care was taken to insure the consumption of the daily dose of the coleus leaves offered. The vitamin feeding was continued for 8 weeks.

RESULTS

Data are presented in table 1 which show that rats receiving, as the sole source of vitamin A in the diet, a daily dose of coleus leaf taken from undivided plants grown entirely in sand gained less during the experimental period than did any other group. Of particular interest is the fact that the average rate of growth of this lot of rats was approximately one-half that of the group given similar-appearing coleus leaf removed from the stunted lateral nourished by the inadequate supply of nutrients present in sand. There were slight differences in the average total gains in body weight made by the three groups of rats fed coleus leaf taken, respectively, from the undivided plant grown in soil, the divided plant grown in sand, and the divided plant grown in soil. Upon analysis, these differences were not found to be statistically significant as the data recorded in table 2 indicate.

TABLE 1.—*Mean weights at end of depletion period and mean gains in 8 weeks of the 4 groups of rats fed vitamin-A-free diets supplemented by 20 mg of coleus leaf taken from plants treated in 3 different ways*

Group	Treatment of plant	Rats		Weight at end of depletion period	Mean-weight gain in 8 weeks
		Started	Survived		
		Number	*Number*	*Grams*	*Grams*
A	Grown in soil	20	17	94	45.82
B	Grown in sand	13	8	98	22.13
C	One-half grown in soil	21	18	99	49.22
D	One-half grown in sand	23	20	103	42.90

TABLE 2.—*Values of t between mean total gains for each series, secured by analysis of variance*

Groups of rats classified according to kind of coleus fed	B, all sand	C, soil half of divided plants	D, sand half of divided plants
A, all soil	[1] 3.015	0.651	0.527
B, all sand		[2] 3.357	[2] 2.448
C, soil half of divided plants			1.122

[1] Significant.
[2] Highly significant.

Estimates of experimental error were obtained by analyzing the variance in the manner devised by Fisher (*3*). Tests of significance for the values of t were made by means of the table of values of r, R, and t adapted by Wallace and Snedecor (*7*) from Fisher's tables. Similar statistical treatment showed that the differences in the mean body weights of the four groups of experimental animals at the end of the depletion period (table 1) were not significant. For instance, in testing for the significance of the differences noted in mean weights of the groups at this time, a value for t of 0.7377 was obtained when groups A and B were compared, and of 1.102 when the mean weights of the rats in groups C and D were similarly analyzed. Inasmuch as these values do not indicate significant differences, the greater increments in growth made by groups A, C, and D in comparison with that made by group B cannot be attributed to any cause except to variation in the vitamin content of the leaves fed to the four groups of animals.

DISCUSSION

These results lead to very interesting speculation. Unmistakably, the leaves of a stunted underdeveloped plant grown in a medium extremely poor in the essential nutrients contain less vitamin A than do the leaves of similar plants grown in a highly favorable nutritive medium. Evidently some factor necessary for the synthesis of vitamin A is lacking in the nutrients provided by the sand. That the lack of certain plant nutrients may definitely retard the synthesis of vitamin A has been noted by Dutcher (*8, p. 123*) in unpublished data. He found that spinach made chlorotic by growing in soil containing insufficient manganese manufactured less vitamin A than did spinach grown with adequate amounts of the element.

On the other hand, a plant, one-half of which is stunted, produces small leaves on the inactively growing side that, weight for weight, are relatively as rich in vitamin A as are the large leaves from the well-nourished half of the plant. The difference in the nutritive state of the two sides of the plant seems to indicate that there is no cross transfer of food materials from one side of the plant to the other in *Coleus blumei*. Apparently, then, the common plant nutrients such as nitrogen, phosphorus, potash, calcium, etc., may not be concerned in the synthesis of the vitamin or the vitamin may have been synthesized and transferred. Auchter (*1*) has shown that water passes from one side of a plant to the other; but water cannot be the determining factor in producing a like vitamin content of the leaves, for the divided and undivided parts were both liberally supplied with water. However, in its passage through the plant, water may have carried from the well-nourished part of the coleus some soluble inorganic or organic material concerned with the manufacture of vitamin A, but not with growth. Or perhaps the vitamin itself or its precursor, carotene, elaborated in the adequately nourished side of the plant was transported to the poorly nourished half. The recent demonstration by Von Euler and Klussmann (*3*) that carotene and other carotenoid pigments pass into aqueous solutions of the bile salts suggests the possibility of a transfer of this nature. The hypothesis of a translocation of vitamin A, however, is not substantiated by certain statements of Hauge and Trost (*4*), if the findings obtained with ears of corn as the test material apply to the present situation. These workers crossed Reid Yellow Dent and Johnson County White Dent corn and were unable to detect any measurable transfer of vitamin A to white endosperm grains selected from F_2 segregating ears. They found that vitamin A was always associated with the yellow endosperm and that white grains when grown on the same ears with the yellow grain did not contain vitamin A.

No reasonable explanation, therefore, can be offered at present for the phenomena described. The most significant point of the experiment lies in the results obtained; i. e., an undivided plant grown in sand does not have the ability to synthesize normal quantities of vitamin A in its leaves whereas a plant, divided and growing one-half in soil and one-half in sand, is able to produce normal quantities of vitamin A in the leaves of the stunted lateral.

SUMMARY AND CONCLUSIONS

Vitamin A was not synthesized in as large quantities in a poorly nourished stunted coleus plant as it was in an actively growing, well-nourished one. However, when differentiated growth was induced in two sides of a single plant, the leaves of the underdeveloped lateral contained relatively as much vitamin as did those on the luxuriantly growing branch.

These findings suggest either a specific but unidentified nutrient factor essential for the synthesis of vitamin A or a cross transfer within the plant of potential vitamin A materials in a manner that is not characteristic of the food factors essential for the growth of the plant.

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THE PHOSPHORUS REQUIREMENTS OF DAIRY HEIFERS[1]

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INTRODUCTION

An earlier paper[3] in this journal dealt with the calcium requirements of growing dairy heifers. Since that report was published an investigation of phosphorus requirements has been conducted along the same general lines.

EXPERIMENTAL ANIMALS

One pure-bred and seven high-grade Holstein-Friesian heifers were used. They were divided into two groups designated as the high-phosphorus and low-phosphorus groups. Table 1 shows their history. Unfortunately, two of them (both in the same group) had to be disposed of when their experimental period was about half completed.

TABLE 1.—*History of the heifers in the phosphorus-requirement tests*

Group	Heifer no.	Born	Age at commencement of experiment	First calf delivered	Age at first calving
			Days		*Days*
High-phosphorus ration	162	Aug. 29, 1929	181	Nov. 28, 1931	821
	175	Jan. 10, 1930	114	June 26, 1932	898
	190	Apr. 12, 1931	159	Sept. 18, 1933	890
	192	Apr. 10, 1931	161	Aug. 29, 1933	872
Average			154		870
Low-phosphorus ration	[1] 163	Aug. 31, 1929	179	No calf	
	[1] 168	Dec. 16, 1929	128	do	
	191	Apr. 12, 1931	159	Aug. 11, 1933	852
	193	Apr. 1, 1931	170	Aug. 19, 1933	871
Average			[2] 165		862

[1] Reacted to the tuberculin test, Feb. 9, 1931; slaughtered Feb. 16, 1931.
[2] Average age of the two that calved.

NATURE OF THE RATIONS

The basal ration was composed of:

Mixed hay (low in phosphorus).
Dried beet pulp.
Grain mixture { 8 parts corn meal. / 1 part corn gluten meal. / 1 part blood flour.

This combination resulted in a ration quite low in phosphorus. For the high-phosphorus group it was supplemented with requisite

[1] Received for publication Mar. 4, 1935; issued August 1935. Contribution no. 188 of the Massachusetts Agricultural Experiment Station.
[2] The advice and suggestions of Dr. J. B. Lindsey, former head of the Department of Chemistry at this station, are acknowledged and appreciated.
[3] LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. THE CALCIUM REQUIREMENTS OF DAIRY HEIFERS. Jour. Agr. Research 42: 883–896, 1931.

amounts of rice bran, a product relatively high in phosphorus. The additional amounts of other nutrients furnished by the rice bran were offset for the low group by slightly larger amounts of the basal ration.

The proportions of the several ingredients fed to the two groups at varying ages are shown in table 2. The slightly larger average daily feed intake by the high-phosphorus group is due to the fact that these animals, as a group, were slightly heavier from start to finish than those in the low-phosphorus group.[4]

The hay fed was designedly as low in phosphorus as could be purchased locally. It was grown on farms in the vicinity of the experiment station, was cut late, and came from run-out fields. The beet pulp was fed to give the ration greater palatability and also to furnish calcium. The grain mixture provided adequate amounts of protein and energy and was very low in phosphorus. The composition of the feeds is shown in table 3.

TABLE 2.—*Average daily feed consumption of heifers by groups and ages*

Group and age	Hay	Beet pulp	Grain mixture	Rice bran	Total daily intake
High-phosphorus:	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Calves	5.63	0.99	3.12	0.99	10.73
Yearlings	9.00	1.28	3.50	1.10	14.88
2-year-olds	11.55	1.88	3.88	1.88	19.19
Low-phosphorus:					
Calves	5.94	.99	3.36	----------	10.29
Yearlings	9.09	1.35	3.77	----------	14.21
2-year-olds	12.72	1.94	3.98	----------	18.64

[4] The method of feeding previous to placing the heifers on the experimental rations at about 5 months of age was the same for all individuals.

TABLE 3.—*Minimum, maximum, and average composition of feeds used in the experiments*

Different lots	Feed	Moisture			Dry matter basis of—																				
					Total ash			Crude protein			Crude fiber			Ether extract			Calcium			Phosphorus			Magnesium		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Number		*Pct.*	*t.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
7	Hay	9.12	10.56	10.10	3.93	6.30	4.98	5.10	7.32	6.44	29.85	33.53	31.83	1.84	2.35	2.06	0.448	0.638	0.511	0.095	0.169	0.134	0.136	0.285	0.185
6	Dried beet pulp	8.03	11.31	9.74	2.50	5.47	3.52	9.56	12.44	10.73	16.13	21.06	19.22	.09	.52	.36	.636	.807	.724	.071	.094	.082	.265	.353	.312
4	Grain mixture	11.83	13.27	12.53	1.47	2.94	2.37	22.57	25.34	23.85	1.85	2.23	1.98	.88	4.59	2.86	.014	.131	.081	.273	.392	.335	.118	.160	.141
5	Rice bran	8.70	9.47	9.13	10.73	12.82	11.76	13.15	15.05	14.19	9.42	10.73	10.03	12.69	17.78	14.31	.043	.131	.082	1.519	1.746	1.611	.698	.858	.757

The high-phosphorus ration supplied about one and two-thirds times as much of that element as did the low-phosphorus ration. Other constituents were kept as nearly on a par as possible (table 4). Intake of digestible protein per unit of body weight was practically identical for both groups throughout, but the high-phosphorus group received an average amount of total digestible nutrients per unit of weight slightly greater than did the low-phosphorus group. This was due to a somewhat higher content of fat in the rice bran than was anticipated. Analyses on record give the average crude fat content of rice bran as about 10 percent; the samples taken during this experiment averaged 14.3 percent.

TABLE 4.—*Daily intake of total and digestible nutrients by heifers, per 100 pounds live weight*

Age	Group	Dry matter	Crude protein	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus	Digestible protein	Total digestible nutrient
		Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
Calves	High-phosphorus	2.10	0.27	0.43	1.25	0.077	0.0085	0.0072	0.17	1.51
	Low-phosphorus	1.96	.26	.39	1.18	.048	.0082	.0040	.16	1.35
Yearlings	High-phosphorus	1.67	.20	.36	.97	.052	.0067	.0050	.12	1.14
	Low phosphorus	1.80	.21	.40	1.07	.041	.0075	.0034	.13	1.22
2-year-olds	High-phosphorus	1.66	.18	.37	.97	.055	.0065	.0045	.10	.91
	Low phosphorus	1.58	.17	.36	.93	.037	.0069	.0026	.10	1.05
Weighted averages for the duration of the experiment.	High-phsophorus	1.78	.22	.38	1.04	.058	.0071	.0055	.13	1.22
	Low-phosphorus	1.71	.21	.37	1.02	.040	.0072	.0033	.13	1.16

RESULTS OF METABOLISM BALANCE TRIALS

Seventy-seven metabolism balance trials were carried out, 42 with the high-phosphorus group and 35 with the low-phosphorus group. It should be borne in mind that these metabolism-balance periods were not continuous, but were conducted at frequent intervals for 10-day periods throughout the course of the investigation. The experimental and analytical procedure was identical with that described in the earlier publication.[5] The progress of the work has been accelerated somewhat by the addition of two more metabolism stalls, thus making it possible to have four heifers on trial at one time. Figure 1 shows the general arrangement of the stalls.

[5] LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

Methods of analysis were as follows:

For nitrogen the Kjeldahl method described in the following publication: ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Compiled by the committee on editing methods of analysis. Ed. 3, 593 pp., illus. Washington, D.C. 1930.

For calcium and magnesium the method of McCrudden modified for use with materials of this nature: MCCRUDDEN, F. H., THE QUANTITATIVE SEPARATION OF CALCIUM AND MAGNESIUM IN PRESENCE OF PHOSPHATES AND SMALL AMOUNTS OF IRON DEVISED ESPECIALLY FOR THE ANALYSIS OF FOODS, URINE AND FECES. Jour. Biol. Chem. 7:83-100. 1910.

For phosphorus the colorimetric method described in the following publication: FISKE, C. H., and SUBBAROW, Y. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66: 387-389. 1925

The frequency with which individual heifers were used as members of the high-phosphorus and low-phosphorus groups was as follows: High-phosphorus group—heifers 162 and 175, 16 times; heifer 190, 4 times; heifer 192, 6 times. Low-phosphorus group—heifer 163, 8 times; heifer 168, 4 times; heifer 191, 11 times; heifer 193, 12 times. The uneven distribution of trials between individuals was due in the first place to the loss of nos. 163 and 168 early in their yearling period, and to a subsequent effort to even up the number of trials for the two

FIGURE 1.—Arrangement of metabolism stalls used in the experiments.

groups by using nos. 191 and 193 considerably more than nos. 190 and 192.

The detailed balance records for the individual heifers are given in table 5 and the data are summarized in table 6. The values in this table were obtained by taking the average of all trials for a given age of all individuals in the group. The probable error of these averages was determined by means of Bessel's formula, viz.,

$$P.E._m = 0.6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

TABLE 5.—*Detailed balance record for each heifer in the high- and low-phosphorus metabolism experiments, 1930–33*

HIGH-PHOSPHORUS GROUP

Heifer no.	Age		Weight	Date of experiment	Daily intake per 100 pounds live weight				Daily retention per 100 pounds live weight				Retention of intake			
					N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg
	Months	*Days*	*Pounds*		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
	6	21	310	Mar. 22 to 31, 1930	22.646	2.897	3.560	2.387	8.345	1.339	1.085	0.072	36.85	46.21	30.48	3.01
	7	21	358	Apr. 19 to 28, 1930	20.358	3.074	3.167	2.228	4.580	1.248	.960	−.197	22.50	40.61	30.31	−8.87
	8	11	395	May 10 to 19, 1930	18.484	2.778	2.882	2.019	5.256	1.160	.978	−.070	28.43	41.77	33.93	−3.46
	9	3	415	June 1 to 10, 1930	17.141	2.756	2.726	1.878	4.844	1.152	.823	−.277	28.26	41.80	30.19	−14.73
Average as a calf					19.657	2.876	3.084	2.128	5.756	1.225	.962	−.118				
	13	7	515	Oct. 5 to 14, 1930	19.054	3.848	3.065	2.242	5.687	.969	.755	.163	29.85	25.17	24.46	7.27
	14	8	580	Nov. 6 to 15, 1930	16.844	3.394	2.758	1.996	4.298	.565	.462	.007	25.52	16.65	16.75	.35
	15	10	625	Dec. 9 to 18, 1930	17.512	3.201	2.861	1.912	5.061	.796	.757	.037	28.90	24.87	26.45	1.95
	16	26	705	Jan. 24 to Feb. 2, 1931	15.772	2.994	2.575	1.763	5.627	.601	.916	−.029	35.68	20.07	35.58	−1.63
	18	6	765	Mar. 7 to 16, 1931	15.132	3.025	2.467	1.742	3.829	.838	.793	.201	25.31	27.69	32.16	11.53
162	18	24	815	Mar. 25 to Apr. 3, 1931	14.354	2.840	2.338	1.643	3.041	.662	.705	.142	21.19	23.33	30.16	8.66
	19	16	835	Apr. 14 to 23, 1931	14.010	2.771	2.282	1.603	2.168	.849	.639	.297	15.47	30.64	27.98	18.55
	20	3	845	May 2 to 11, 1931	14.529	2.997	2.343	1.677	2.779	.551	.609	.036	19.13	18.38	26.01	2.16
	20	22	875	May 21 to 30, 1931	13.893	2.854	2.246	1.604	2.892	.954	.406	.057	20.82	33.43	18.06	3.58
	21	10	900	June 8 to 17, 1931	13.474	2.726	2.149	1.529	1.678	.938	.345	.037	12.45	34.46	16.05	2.41
Average as a yearling					15.457	3.065	2.510	1.771	3.706	.772	.639	.095				
	25	4	960	Oct. 3 to 12, 1931	14.303	3.409	2.766	1.784	3.763	1.196	.538	.140	26.31	35.09	19.45	7.86
	26	9	1,005	Nov. 7 to 16, 1931	13.753	3.306	2.648	1.715	4.568	1.619	.615	−.102	33.21	48.96	23.22	−5.95
Average as a 2-year-old					14.028	3.358	2.707	1.750	4.166	1.408	.577	.019				
	9	10	495	Oct. 20 to 29, 1930	17.561	3.587	2.971	2.149	4.649	1.372	.520	.028	26.47	38.25	17.51	1.32
	10	14	565	Nov. 24 to Dec. 3, 1930	17.127	3.171	2.907	1.939	5.393	1.124	1.202	.233	31.49	35.45	41.34	12.04
Average as a calf					17.344	3.379	2.939	2.044	5.021	1.248	.861	.131				
	12	0	600	Jan. 10 to 19, 1931	16.218	3.023	2.748	1.836	4.313	.735	1.042	.205	26.59	24.33	37.92	11.17
	12	29	650	Feb. 8 to 17, 1931	15.095	2.773	1.676	1.295	3.087	.527	.668	−.423	20.45	19.00	39.87	−32.64
	13	25	710	Mar. 7 to 16, 1931	15.621	2.992	2.560	1.777	2.931	.683	.645	.102	18.76	22.84	25.19	5.71
	14	15	745	Mar. 25 to Apr. 3, 1931	15.051	2.852	2.465	1.702	2.827	.989	.778	.144	18.79	34.67	31.57	8.46
	15	4	765	Apr. 14 to 23., 1931	14.657	2.777	2.400	1.658	2.487	.672	.755	.293	16.97	24.19	31.47	17.65
	15	22	790	May 2 to 11, 1931	14.917	2.962	2.417	1.703	2.691	.355	.511	.013	18.04	11.98	21.16	.77
	16	11	820	May 21 to 30, 1931	14.232	2.814	2.312	1.625	3.206	1.358	.619	.114	22.53	48.27	26.75	7.01
175	16	29	840	June 8 to 17, 1931	13.871	2.700	2.222	1.556	3.008	.314	.206	.013	21.69	11.61	9.26	.81

	20	23	870	Oct. 3 to 12, 1931	15.101	3.376	2.973	1.866	3.161	1.132	.691	.212	20.93	33.54	23.25	11.37
	21	28	925	Nov. 7 to 16, 1931	14.291	3.223	2.801	1.765	3.437	1.424	.293	−.065	24.05	44.20	10.45	−3.68
	23	16	1,030	Dec. 26, 1931, to Jan. 4, 1932	13.921	3.510	2.670	1.744	2.971	.819	.221	.015	21.34	23.34	8.28	−.86
Average as a yearling					14.816	3.000	2.477	1.684	3.102	.819	.584	.057				
	25	10	1,120	Feb. 20 to 29, 1932	12.796	2.724	2.427	1.837	2.437	.106	.355	−.028	19.04	3.88	14.62	−1.53
	26	23	1,190	Apr. 2 to 11, 1932	12.874	2.996	2.356	1.908	2.719	.618	.291	.171	21.12	20.63	12.34	8.96
	27	15	1,240	Apr. 23 to May 4, 1932	12.431	2.900	2.256	1.831	2.410	.605	.054	.040	19.39	20.86	2.38	2.16
Average as a 2-year-old					12.700	2.873	2.346	1.859	2.522	.443	.233	.061				
	6	8	340	Oct. 20 to 29, 1931	25.670	5.241	4.379	2.844	7.482	2.036	1.494	−.200	29.15	38.84	34.12	−7.04
	7	16	405	Nov. 28 to Dec. 7, 1931	21.494	4.422	3.677	2.385	5.531	2.403	1.032	−.121	25.73	54.35	28.07	−5.07
	9	11	490	Jan. 23 to Feb. 1, 1932	20.319	3.614	3.289	2.451	4.850	1.193	1.675	.146	23.87	33.02	50.93	5.96
190	11	7	568	Mar. 19 to 28, 1932	18.317	3.490	2.945	2.255	3.591	.690	.555	−.159	19.60	19.76	18.84	−7.04
Average as a calf					21.450	4.192	3.573	2.484	5.364	1.581	1.189	−.084				
	6	10	390	Oct. 20 to 29, 1931	23.503	5.235	3.977	2.622	6.858	1.646	1.038	−.132	29.18	31.45	26.09	−5.04
	7	18	476	Nov. 28 to Dec. 7, 1931	20.060	4.503	3.404	2.240	6.172	2.094	.772	−.068	30.77	46.50	22.69	−3.01
	8	16	495	Dec. 26, 1931, to Jan. 4, 1932	21.069	4.725	3.413	2.250	6.573	2.029	.882	.051	31.19	42.94	25.85	2.25
192	9	13	545	Jan. 23 to Feb. 1, 1932	19.120	3.647	3.071	2.354	4.726	1.038	1.359	.091	24.72	28.45	44.24	3.87
	10	10	585	Feb. 20 to 29, 1932	17.771	3.382	2.850	2.186	4.651	.569	.922	−.036	26.17	16.84	32.36	−1.67
	11	9	616	Mar. 19 to 28, 1932	16.984	3.548	2.759	2.184	3.875	.729	.903	−.141	22.82	20.54	32.73	−6.47
Average as a calf					19.751	4.173	3.246	2.306	5.476	1.351	.979	−.039				

LOW-PHOSPHORUS GROUP

	6	19	315	Mar. 22 to 31, 1930	20.605	3.427	1.643	1.624	6.407	0.676	0.522	−.482	31.10	19.72	31.77	−29.65
	7	19	365	Apr. 19 to 28, 1930	18.532	3.515	1.510	1.566	4.184	.621	.565	−.262	22.58	17.66	37.44	−16.71
	8	9	410	May 10 to 19, 1930	16.518	3.121	1.346	1.390	3.846	.037	.383	−.249	23.28	1.20	28.42	−17.89
	9	1	430	June 1 to 10, 1930	15.300	3.077	1.266	1.280	4.967	.971	.498	−.416	32.46	31.55	39.37	−32.52
Average as a calf					17.739	3.285	1.441	1.465	4.851	.576	.492	−.352				
163																
	13	5	545	Oct. 5 to 14, 1930	17.719	3.940	1.862	1.733	4.588	1.019	.610	.092	25.89	25.87	32.75	5.28
	14	6	600	Nov. 6 to 15, 1930	16.196	3.562	1.699	1.574	4.250	.594	.444	−.067	26.24	16.67	26.15	−4.23
	15	9	635	Dec. 9 to 18, 1930	17.102	3.377	1.941	1.537	4.649	.644	.512	−.047	27.18	19.07	26.40	−3.04
	16	24	695	Jan. 24 to Feb. 2, 1931	15.848	3.248	1.791	1.463	.892	.609	.846	−.105	5.63	18.76	47.25	−7.18
Average as a yearling					16.716	3.532	1.823	1.577	3.595	.717	.603	−.032				

TABLE 5.—*Detailed balance record for each heifer in the high- and low-phosphorus metabolism experiments, 1930–33*—Continued

LOW-PHOSPHORUS GROUP—Continued

Heifer no.	Age		Weight	Date of experiment	Daily intake per 100 pounds live weight				Daily retention per 100 pounds live weight				Retention of intake			
					N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg
	Months	*Days*	*Pounds*		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
	10	4	430	Oct. 20 to 29, 1930	18.143	3.621	1.859	1.664	4.565	.863	.738	−.060	25.16	23.82	39.72	−3.63
	11	8	495	Nov. 24 to Dec. 3, 1930	17.484	3.168	1.921	1.494	6.146	.994	.965	.201	35.15	31.38	50.25	13.45
Average as a calf					17.814	3.395	1.890	1.579	5.356	.929	.852	.071				
168																
	12	25	520	Jan. 10 to 19, 1931	16.729	3.052	1.842	1.433	4.989	.962	.971	.109	29.82	31.52	52.72	7.60
	13	23	565	Feb. 8 to 17, 1931	17.366	3.190	1.928	1.490	3.552	.606	.768	−.486	20.45	19.00	39.87	−32.64
Average as a yearling					17.048	3.121	1.885	1.462	4.271	.784	.870	−.189				
	6	8	390	Oct. 20 to 29, 1931	23.040	5.170	2.312	1.796	8.026	1.874	.796	−.081	34.83	36.24	34.43	−4.51
	7	16	455	Nov. 28 to Dec. 7, 1931	19.700	4.457	1.986	1.539	6.572	2.227	.748	−.036	33.36	49.98	37.65	−2.33
	8	14	490	Dec. 26, 1931 to Jan. 4, 1932.	20.075	4.702	2.043	1.580	6.267	1.993	.847	.029	31.22	42.38	41.46	1.83
	9	11	540	Jan. 23 to Feb. 1, 1932	18.176	3.615	1.817	1.743	4.736	1.086	.853	−.094	26.06	30.08	46.94	−5.38
	10	8	575	Feb. 20 to 29, 1932	17.043	3.380	1.706	1.635	3.675	.306	.812	−.109	21.56	9.04	47.59	−6.67
	11	21	620	Apr. 2 to 11, 1932	16.346	3.451	1.658	1.629	2.607	.695	.334	−.064	15.95	20.13	20.15	−3.92
Average as a calf					19.063	4.129	1.920	1.654	5.314	1.364	.732	−.059				
191																
	12	13	664	Apr. 25 to May 4, 1930	15.443	3.267	1.572	1.539	3.720	1.005	.441	.065	24.09	30.77	28.06	4.25
	13	10	682	May 22 to 31, 1932	15.178	3.166	1.535	1.503	3.310	.607	.286	.132	21.81	19.16	18.63	8.77
	14	0	695	June 12 to 21, 1932	14.823	3.021	1.495	1.445	2.765	.723	.529	.036	18.65	23.92	35.43	2.46
Average as a yearling					15.148	3.151	1.534	1.496	3.265	.778	.419	.078				
	24	2	1,011	Apr. 14 to 23, 1933	12.111	2.947	1.143	1.315	2.277	.634	.372	−.096	18.80	21.52	32.56	−7.28
	25	3	1,073	May 15 to 24, 1933	12.736	3.193	1.209	1.413	2.221	1.204	.280	.106	17.44	37.70	23.20	7.47
Average as a 2-year old					12.424	3.070	1.176	1.364	2.249	.919	.326	.005				
	6	19	405	Oct. 20 to 29, 1931	22.187	4.979	2.226	1.729	7.333	2.463	.942	−.209	33.05	49.48	42.34	−12.11
	7	27	470	Nov. 28 to Dec. 7, 1931	19.072	4.314	1.923	1.490	5.910	1.807	.787	−.175	30.98	41.89	40.93	−11.74
	8	25	510	Dec. 26, 1931 to Jan. 4, 1932.	19.288	4.518	1.963	1.518	5.732	2.471	.889	−.205	29.72	54.69	45.31	−13.49
	9	22	540	Jan. 23 to Feb. 1, 1932	17.970	3.519	1.789	1.707	4.107	1.128	.875	−.160	22.85	32.05	48.92	−9.35
	10	19	580	Feb. 20 to 29, 1932	16.896	3.351	1.691	1.621	3.639	.728	.888	−.104	21.54	21.73	52.50	−6.39

Average as a calf					19.083	4.136	1.918	1.613	5.344	1.719	.876	−.171				
193	12	1	640	Apr. 2 to 11, 1932	15.835	3.343	1.606	1.578	2.876	.981	.413	−.106	18.16	29.34	25.74	−6.72
	12	24	675	Apr. 25 to May 4, 1932	15.191	3.214	1.547	1.514	2.825	.952	.303	−.006	18.60	29.61	19.61	−.37
	13	21	693	May 22 to 31, 1932	14.937	3.116	1.511	1.479	3.037	.975	.420	.010	20.33	31.30	27.77	.67
	14	11	710	June 12 to 21, 1932	14.510	2.957	1.463	1.415	2.536	.782	.523	−.016	17.48	26.44	35.71	−1.14
Average as a yearling					15.118	3.158	1.532	1.497	2.819	.923	.415	−.030				
	24	0	995	Mar. 27 to Apr. 5, 1933	12.669	3.003	1.193	1.349	2.078	.542	.245	−.124	16.40	18.05	20.56	−9.19
	24	28	1,061	Apr. 29 to May. 8, 1933	12.705	3.169	1.205	1.405	2.648	1.035	.433	−.017	20.84	32.67	35.91	−1.21
	26	2	1,130	June 3 to 12, 1933	12.065	3.024	1.144	1.338	1.748	1.024	.292	−.014	14.49	33.85	25.53	−1.04
Average as a 2-year-old					12.480	3.065	1.181	1.364	2.158	.867	.323	−.052				

TABLE 6.—*Summary of balances by ages in the high- and low-phosphorus metabolism experiments, March 1930 to June 1933*

Group and number of trials	Daily intake, per 100 pounds live weight				Daily retention, per 100 pounds live weight				Retention of intake				Ratio of Ca to P	
	N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg	Intake	Retention
High-phosphorus:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*		
As calves, 16 trials	19.85	3.75	3.25	2.27	5.46 ±.22	1.36 ±.09	1.01 ±.05	−0.05	27.51 ±.72	36.27 ±1.77	31.08 ±1.48	0	1.16:1	1.35:1
As yearlings, 21 trials	15.12	3.03	2.49	1.73	3.39 ±.16	.80 ±.04	.61 ±.03	.07	22.42 ±.78	26.40 ±1.38	24.50 ±1.33	4.05	1.22:1	1.31:1
As 2-year-olds, 5 trials	13.23	3.07	2.49	1.82	3.18 ±.43	.83 ±.26	.37 ±.10	.04	24.04 ±2.70	27.04 ±7.66	14.86 ±3.58	2.20	1.23:1	2.24:1
Average	16.70	3.31	2.78	1.94	4.15 ±.16	1.02 ±.05	.73 ±.04	.02	24.85 ±.57	30.82 ±1.21	26.26 ±1.05	1.03	1.19:1	1.38:1
Low-phosphorus:														
As calves, 17 trials	18.61	3.85	1.80	1.59	5.22 ±.24	1.23 ±.12	.73 ±.03	−.15	28.05 ±.94	31.95 ±2.43	40.56 ±1.42	0	2.13:1	1.68:1
As yearlings, 13 trials	15.91	3.27	1.68	1.52	3.38 ±.21	.80 ±.03	.54 ±.04	−.03	21.24 ±1.14	24.46 ±1.04	32.14 ±1.89	0	1.95:1	1.48:1
As 2-year-olds, 5 trials	12.46	3.07	1.18	1.36	2.19 ±.15	.89 ±.13	.32 ±.03	−.03	17.58 ±1.08	28.99 ±3.82	27.12 ±2.90	0	2.60:1	2.78:1
Average	16.73	3.52	1.67	1.53	4.11 ±.19	1.02 ±.07	.60 ±.03	−.09	24.57 ±.77	28.98 ±1.29	35.93 ±1.14	0	2.11:1	1.70:1

RETENTION AS RELATED TO INTAKE

Considering first the results as a whole: The nitrogen, calcium, and phosphorus balances were positive in all cases. With the average intake of nitrogen per unit of weight practically identical for both groups, the high-phosphorus group retained 1 percent more, indicating slightly, but not significantly, better utilization of nitrogen by that group.

Retention of calcium per unit of weight was the same for both groups, but since the unit intake for the high-phosphorus group was 6 percent less than for the low-phosphorus group, utilization by the former was slightly, but not significantly, superior (30.8 percent as contrasted with 29 percent).

Retention of phosphorus per unit of weight was somewhat greater in the high-phosphorus group (0.73 g as compared with 0.60 g daily), but since the intake by the low-phosphorus group was only 60 percent that of the high-phosphorus group, the utilization by the low-phosphorus group was significantly greater (35.9 percent as contrasted with 26.3 percent). This is in agreement with the earlier work on calcium requirements,[6] where it was noted that the lower intake was more efficiently utilized.

Forty-one of the magnesium balances were negative; 15 in the high-, 26 in the low-phosphorus group. Although they occurred more frequently in the low-phosphorus group, the average negative balance was not much larger for that group (−0.146 g daily per 100 pounds live weight as compared with −0.137 g daily for the high-phosphorus group). The net result for the groups as a whole was a very slight positive balance of magnesium for the high-phosphorus group and a slight negative balance of that element for the low-phosphorus group. The significance of such a large number of negative balances of an element is not readily apparent. A similar situation was noted and commented upon in the earlier paper on the subject.[7] The departure from normal environment in the conduct of metabolism experiments, while it may not interfere to any extent with storage of elements which are utilized in relatively large proportion to their intake (e. g., nitrogen, calcium, or phosphorus), may be sufficient at times to shift to the negative side the equilibrium of an element like magnesium, which at best is utilized in relatively small proportion.

EFFECT OF AGE ON INTAKE, RETENTION, AND UTILIZATION

The average results [8] for different ages are portrayed graphically in figures 2 and 3 in addition to being summarized in table 6. The graphs show the relative decrease (or increase) in intake, retention, and percentage utilization per unit of weight with advancing age. They were constructed by assuming in each case a value of 100 for the first year, values for the second and third years being expressed in the appropriate percentage ratio. They show quite clearly—

(1) That intake per unit of weight in general decreased with advancing age, which is normal. The rate of decrease was usually more

[6] LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.
[7] LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.
[8] Because of the many negative balances, results for magnesium do not lend themselves to graphic portrayal, and hence are not included.

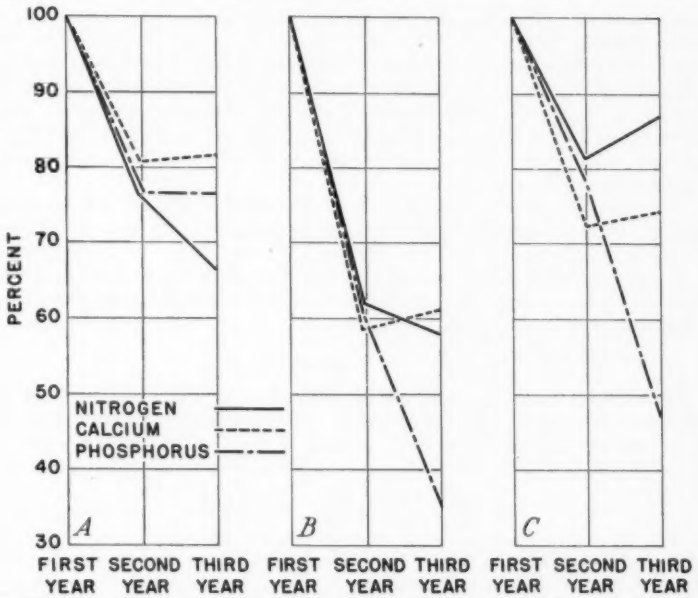

FIGURE 2.—Rate of decrease or increase in intake (*A*), retention (*B*), and percentage utilization (*C*) of nitrogen, calcium, and phosphorus by the high-phosphorus group of heifers as affected by increasing age.

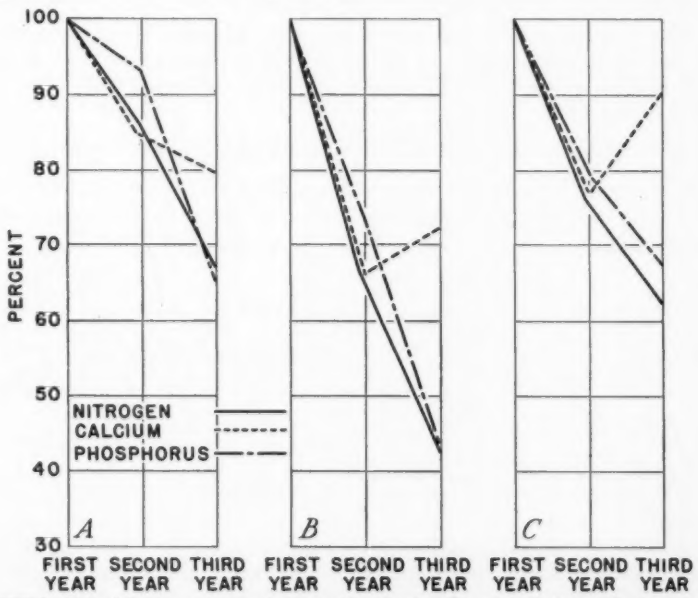

FIGURE 3.—Rate of decrease or increase in intake (*A*), retention (*B*), and percentage utilization (*C*) of nitrogen, calcium, and phosphorus by the low-phosphorus group of heifers as affected by increasing age.

rapid from the first year of life to the second than from the second to the third.

(2) That retention, per unit of weight, of all three elements decreased rapidly from the first year of life to the second, and, in the case of calcium, increased slightly from the second to the third year, the increase being more marked in the low-phosphorus group. Nitrogen and phosphorus retention continued to decrease in both groups during the third year.

(3) That in general percentage utilization (ratio of retention to intake), was roughly parallel to actual retention in its rate of decrease for both groups and the three elements.

The principal point of interest here is the upswing in retention and utilization of calcium in the third year for both groups, possibly a reflection of the demands of pregnancy.

CALCIUM PHOSPHORUS RATIO

Respecting ratio of calcium to phosphorus fed and retained no definite conclusions can be drawn. The tendency in retention has been toward a general level at around 1.5:1. The high-phosphorus group, with a somewhat lower average intake ratio, had an average retention ratio of 1:1.4, while the low-phosphorus group, with a considerably higher average intake ratio, leveled down on retention to 1:1.7. The exceptions to this statement are the retention ratios for 2-year-olds in both groups. It is thought that possibly the marked departures in these cases are due to the relatively small amount of data for that age group.

GROWTH AND REPRODUCTION RECORDS OF THE ANIMALS

The procedure for recording growth was the same as in the earlier work.[3] A graphic summary of the growth records appears in figure 4. Table 7 gives a record of the weight and condition at birth of each heifer's first calf.

TABLE 7.—*Weight and condition at birth of calves born to heifers in both phosphorus groups* [1]

Calf of heifer no.—	High-phosphorus group		Calf of heifer no.	Low-phosphorus group	
	Weight at birth	Condition at birth		Weight at birth	Condition at birth
	Pounds			*Pounds*	
162	[2] 40	Good.	[3] 163	([4])	
175	95	Do.	[3] 168	([4])	
190	96	Do.	[5] 191	85	Good.
192	98	Excellent.	193	100	Excellent.
Average	96.3		Average	92.5	

[1] In all cases only one service was required for conception.
[2] Sired by a Jersey bull; not included in the average.
[3] As previously noted, these animals had to be disposed of as yearlings.
[4] No calf.
[5] Retained the placenta.

The graphs reveal a quite uniform rate of growth. The low phosphorus group averaged smaller at the start and, with respect to girth,

[3] LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

they continued so throughout the experiment. Their average weights were less until within a month before the experiment ended. Their superiority in height after the nineteenth month is attributed to the fact that data were available for only two heifers in this group (191 and 193) during the latter half of the experiment. This is confirmed by plotting the height curves for these two only for the entire experi-

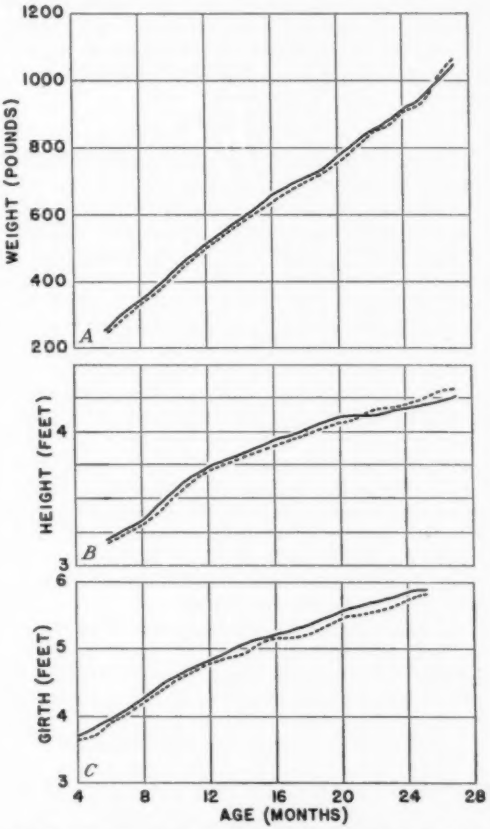

FIGURE 4.—Composite curves representing average weights (*A*), height at withers (*B*), and heart girths (*C*) of heifers in the high- and low-phosphorus groups. The solid lines represent the high-phosphorus and the broken lines the low-phosphorus groups. Commencing with the fourteenth month the curve for the low-phosphorus group is a composite for two animals only.

ment, and comparing them with the curves for their mates in the high-phosphorus group (190 and 192) also for the entire experiment. When thus segregated, the average height for the two in the low-phosphorus group is seen to have been greater throughout than that of their mates in the high-phosphorus group, indicating an obvious reason for change in the trend of the composite graph in the latter part of the period.

Differences in the evidence recorded in table 7 regarding the effect of the rations on the reproductive function are so slight that they are probably without significance.

In addition to this evidence secured while the heifers were on the experimental rations it is pertinent to include the fact that their subsequent history as cows with regard to either production or reproduction does not indicate that the high-phosphorus is superior in any way to the low-phosphorus group. If anything, the latter group has a more satisfactory record. It is possible, of course, that benefits from the larger amount of phosphorous stored by the high-phosphorus group may be apparent later on, that these individuals may have a longer productive life, or that they may be able to stand the strain of continued high production better than the low-phosphorus group.

DISCUSSION

From the data here presented it seems reasonable to conclude that heifers can make average growth on rations supplying amounts of phosphorus similar to those supplied by the low-phosphorus ration, that is, 1.8 g of phosphorus daily per 100 pounds of live weight during the first year of life, 1.7 g during the second year, and 1.2 g during the third year. That these amounts represent the optimum is not contended; probably they are somewhere close to the minimum. Hay containing less than 0.20 percent of phosphorus and consumed in normal amounts will not supply these amounts of phosphorus. Rowen and legume hays contain sufficiently more than that amount (0.20 percent) to provide a reasonable margin of safety even if nothing else is fed, but the average of a large number of analyses of ordinary mixed hays grown in Massachusetts shows only 0.16 percent of phosphorus, while some samples have been found that run as low as 0.10 percent. Where the roughage consists entirely of ordinary mixed hay, and especially where the quality is inferior, some other source of phosphorus should be supplied. The most logical and practicable way to make up possible deficiencies in this respect is through limited grain feeding, which is nothing more than most successful feeders do regularly.

The lowest phosphorus carrier among our common grains is corn, but even this will supply the deficiency when fed at a level of 3 pounds daily, while much smaller amounts of such high phosphorus carriers as wheat bran or linseed meal will supply the necessary amount.

SUMMARY AND CONCLUSIONS

In summarizing the results attention is called to the following points:

Retention and utilization of nitrogen and calcium were of a similar magnitude in both high- and low-phosphorus groups, such small differences as existed not being of statistical significance, except in the case of nitrogen storage by the 2-year-old subgroups. The apparently significant difference here may have been due, however, to an insufficient amount of data in these age groups.

Retention of phosphorus by the high-phosphorus group was superior at all ages, and significantly so during the first year. The low-phosphorus group made better use of the phosphorus they received, but not sufficiently so to equal the high-phosphorus group in phosphorus storage.

Differences in growth and reproductive function were very slight

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